Inflammation and breast cancer
Clinical markers and impact on breast cancer incidence, severity and survival

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Inflammation and breast cancer:

Clinical markers and impact on breast cancer incidence, severity and survival

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Abstract

This thesis investigated whether inflammation is implicated in breast cancer aetiology and survival. For this purpose, circulating markers of inflammation and inflammatory clinical disorders were studied in relation to the risk, severity, and survival of breast cancer in a large Swedish cohort, the Apolipoprotein MORtality RISk Study (AMORIS), which includes >800,000 participants in Greater Stockholm area.

Common inflammatory markers: **serum C-reactive protein (CRP), albumin, haptoglobin and white blood cells (WBC)** were examined in relation to breast cancer risk and survival using Cox proportional hazard regression models. Proportional odds models were employed to assess these markers with regards to breast cancer severity. Systemic inflammation was shown to be weakly associated with breast cancer risk and survival.

Allergy, which has been increasingly linked to cancer in part through inflammation, was also evaluated using **serum allergen-specific IgE** against inhalant allergens**.** Overall, serum specific IgE was inversely associated with the risk of cancer particularly in women. A similar but weaker trend was seen for breast cancer..

Serum lactate dehydrogenase (LDH), a marker of inflammation and metabolic alterations in cancer, was studied in relation to cancer survival. Among breast cancer patients, women with higher serum LDH were associated with worse overall survival, suggesting its relevance in breast cancer growth and progression.

Associations between components of metabolic syndrome, which has often been linked to inflammation, and breast cancer survival were evaluated using prediagnostic **serum glucose, triglycerides, and total cholesterol**. In a competing risk analysis using latent class proportional hazard models, this association differed by patients characteristics, indicating a complex link where competing outcomes are involved.

In summary, findings derived from this thesis contribute to a further understanding of the role of inflammation in breast cancer, and may provide directions towards future mechanistic and clinical research.

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List of Abbreviations

AGE	Advanced Glycation End product
AJCC	American Joint Committee on Cancer
AMORIS	Swedish Apolipoprotein MORtality RISk cohort
APC	Antigen Presenting Cell
BC	Breast Cancer
BCR	B-Cell Receptor
BCS	Breast conservation surgery
BMI	Body Mass Index
BRCA1/2	BReast CAncer GENE 1 and 2
CALAB	Swedish Central Automation Laboratory
CCI	Charlson Comorbidity Index
CI	Confidence Interval
CNS	Central Nervous System
CRP	C-reactive protein
CTL	Cytotoxic T-cell
CV	Cardiovascular disease
DC	Dendritic cell
DCIS	Ductal Carcinoma In Situ
DES	Diethylstilbestrol
DLBCL	Diffuse Large B-Cell Lymphoma
ELISA	Enzyme-Linked ImmunoSorbent Assay
ER	Oestrogen-Receptor
HDL-C	High-Density Lipoprotein Cholesterol
HER2	Human Epidermal Growth Factor-2
HL	Hodgkin Lymphoma
HR	Hazard Ratio
HRT	Hormone Replacement Therapy
hs-CRP	High sensitivity C-reactive protein
ICD	International Classification of Diseases
IPI	International Prognostic Index
IFN	Interferon
IgA	Immunoglobulin A

IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgG4	Immunoglobulin G4
IgE	Immunoglobulin E
IgM	Immunoglobulin M
IGF-1	Insulin-like Growth Factor 1
IL	Interleukin
LCIS	Lobular Carcinoma In Situ
LDH	Lactate Dehydrogenase
LDL-C	Low-Density Lipoprotein Cholesterol
MDSC	Myeloid-Derived Suppressor Cell
MRI	Magnetic Resonance Imaging
mTOR	Mammalian Target of Rapamycin
NF-$\varkappa$B	Nuclear Factor Kappa Beta
NHL	Non Hodgkin Lymphoma
NHS	National Health Service
NK	Natural Killer cell
NMSC	Non Melanoma Skin Cancer
OR	Odds Ratio
PR	Progesterone-Receptor
PRR	Pattern Recognition Receptor
PTEN	Phosphatase and Tensin homolog
RBC	Red Blood Cell
Ref	Referent category
RNI	Reactive Nitrogen Intermediate
ROS	Reactive Oxygen Species
SD	Standard Deviation
SES	Socioeconomic Status
SLNB	Sentinel Lymph Node Biopsy
SOCS3	Suppressor of Cytokine Signalling 3
STAT3	Signal Transducer and Activator of Transcription 3
T_H1	Type 1 T-helper cell
T_H2	Type 2 T-helper cell

TC	Total Cholesterol
TCR	T-Cell Receptor
TG	Triglycerides
TGF-β	Transforming Growth Factor-β
TLR	Toll-Like Receptor
TNF-α	Tumour Necrosis Factor-α
TNM	Tumour, Nodes, Metastasis staging system
TP53	Tumour Protein-53
UK	United Kingdom
USA	United States of America
WBC	White blood cell
WHO	World Health Organisation

Chapter 1: Introduction

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer-specific death in females worldwide (1). In 2012, it was estimated that 1.7 million women were diagnosed with breast cancer and nearly 522,000 died from the disease. Despite rising incidence rates in developing countries during the past two decades (2), about half of all the breast cancer cases and 40% of the deaths still occur in more developed countries (1, 3, 4). Consequently, these trends also imply that by 2020, most of breast cancer deaths will occur in countries with poor resources for healthcare (5). In addition, there is persisting discrepancy in the proportions of breast cancer survival across the globe even among similarly developed countries (6). Among all cancers in the European Union, breast cancer accounts for the highest health care expenditure, with a total of over €6.7 billion or 13% of all cancer-related health care cost in 2009 (7). Besides inflicting major economic burden, breast cancer diagnosis and recurrence cause a deterioration in patients' quality of life, which in turn affects their overall survival (8, 9). Personalised medicine has become a promising strategy to enhance favourable outcomes for breast cancer, however, its benefit is limited to cancers expressing known biological targets (10, 11). Moreover, there is a growing issue of therapy resistance partly due to alternative activation of cancer-promoting mechanisms (12). Therefore, comprehensive understanding of biological pathways involved in breast cancer development and progression as well as factors affecting them is imperative for better utilisation of biological markers for breast cancer intervention.

Inflammation has been acknowledged as one of the mechanisms leading to cancer and its progression (13), but its role in breast cancer development is unclear. Adding to this complexity, inflammation is characteristically found in an array of clinical disorders such as atopy and metabolic disorders, which have also been linked to cancer (14, 15). To gain further insight into the intricate relationship between inflammation and breast cancer, clinical markers of inflammation, atopy and metabolic disorders were used here to explore the role of inflammation and its underlying factors in breast cancer risk, severity and survival using data from the Swedish Apolipoprotein Mortality Risk (AMORIS) cohort.

In order to fully understand the scope of breast cancer risk prediction, severity and survival, and how inflammation may be implicated in these processes, it is important to briefly review current evidence on breast cancer and inflammation, and potential mechanisms underlying their association as discussed in the following section.

1.1. Breast Cancer

1.1.1. Biology of breast cancer

1.1.1.1. The normal breast

The adult breasts lie horizontally atop the pectoral muscle and ribcage. Anatomically, each breast extends from below the clavicle to the centre of the axilla and across to the edge of sternum (16). The outer layers of the breast are formed by the skin, with areola and nipple at the centre, and underlying fat tissue with embedded blood and lymph vessels and nerves (Figure 1). The intrinsic structures of the breast reflect its primary function: to produce milk for lactation. Inner breast consists of two main components: the mammary glands and supporting stroma. Primordial mammary glands are derived from ectoderm in early of embryonic life and differentiate into ductal and lobular-alveolar structures which are present at birth (17, 18). Although breasts of male and female newborns are alike, female breasts undergo structural and physiological changes later in life under the influence of female sex hormones (19). The development of the ductal system, stromal tissue and fat deposition are promoted by estrogens, whereas the development of the lobules and alveoli are mainly stimulated by progesterone. However, neither both hormones promote lactation, which occurs upon stimulation by prolactin (20, 21).

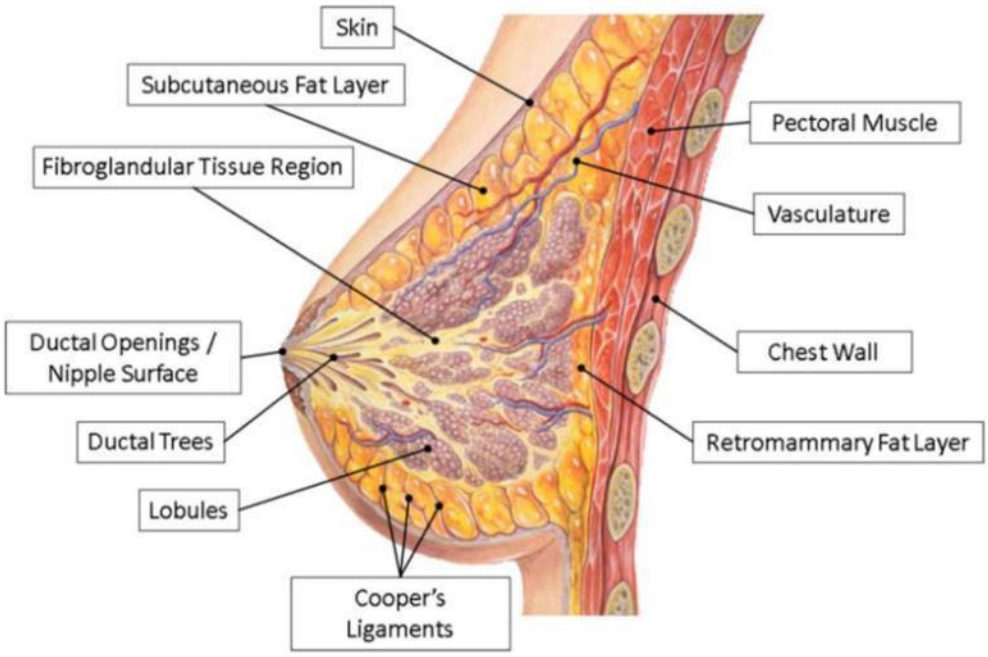


Figure 1. Structure of the human breast (22, 23)

Vasculature of the breast, in particular lymph drainage, is important because of its role in cancer metastasis. Lymph passes from the nipple, areola, and lobules to the subareolar lymphatic plexus. From here, most (>75%) lymph drains to the axillary lymph node, and continues to the clavicular (infraclavicular and supraclavicular) lymph nodes (Figure 2). Lymph may also drain directly to the clavicular lymph nodes, or to the parasternal lymph nodes, the abdominal lymph nodes, and the opposite breast (16).

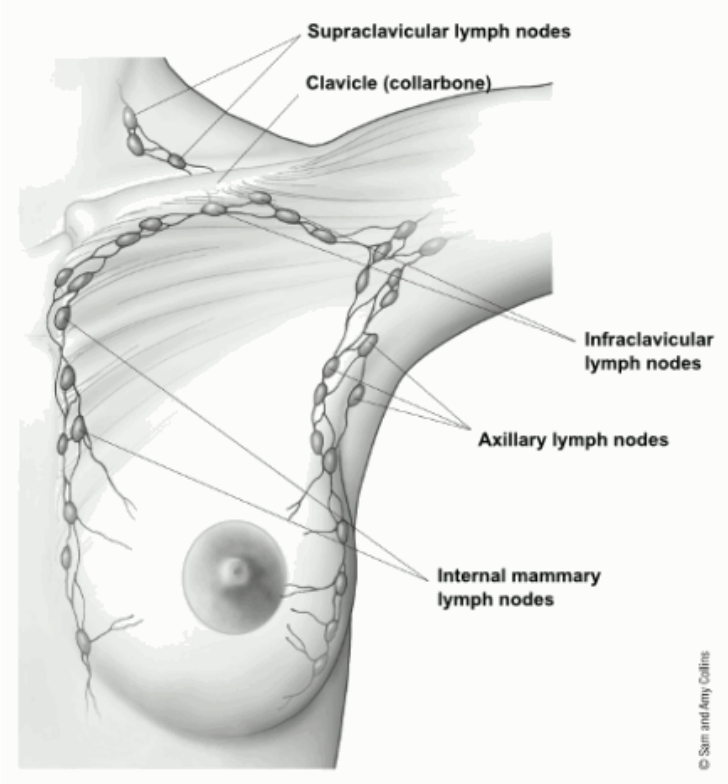


Figure 2. Lymph vasculature related to the breast (24)

1.1.1.2. Histopathology of breast cancer

Breast cancer is the malignant tumour that originates in the cells of the breast (25). It is more common in the left breast and about half occur in the upper outer quadrant. Pathological classification of breast cancer depends on a number of characteristics of the tumour, but largely it is based on the cells where it originates from and invasiveness of the tumour. More than 90% of breast cancers arise from the ducts and are known as ductal carcinomas, and most of the remaining are lobular carcinomas which originate in the lobules (26). When the tumour is confined within the ductal or the lobular basement membrane, it is classified as non-invasive cancer, or more widely known as 'carcinoma *in situ*'. Ductal carcinoma in situ (DCIS) comprises the majority of non-invasive breast cancer, followed by lobular carcinoma in situ (LCIS). Both

DCIS and LCIS may progress into invasive breast cancer, where malignant cells invade surrounding stromal tissue, lymphatic and vascular spaces. Among all breast cancers, invasive ductal carcinoma makes up around 75% of all cases, whereas invasive lobular carcinoma accounts for 5-10%. Although most invasive ductal carcinomas are of 'no special type', a small proportion has specific histopathological features and thus falls into the category of 'special type', which includes medullary carcinoma, tubular carcinoma, mucinous carcinoma, and Paget's disease of the breast (26, 27).

Although histological features of invasive breast cancer cells may resemble those of carcinoma *in situ*, a large discrepancy exists between the two types with regards to clinical management and outcomes. This thesis focuses on invasive breast cancer, which is discussed in more detail below.

1.1.1.3. Invasive breast cancer

According to the World Health Organization's (WHO) classification of breast tumours, invasive breast cancers are characterised by an invasion to adjacent tissue and a tendency to metastasise to distant sites (28). As mentioned above, most invasive breast cancers are of ductal origin, among which the majority is of no special type (NOS). Besides cell types, other tumour characteristics or markers have been utilised in the attempt to better envisage clinical outcomes of invasive breast cancer. The terms 'prognostic' and 'predictive' have often been used in describing these markers, and it is important to understand the difference between the two, although a marker may fall into both categories. A prognostic factor is described as 'a measurement that is associated with clinical outcome in the absence of therapy or with the application of a standard therapy that patients are likely to receive', which is often seen as the natural history of the disease. On the other hand, a predictive factor is 'a measurement associated with a response or lack of response to a particular therapy', and is considered essential in depicting the interaction between such a marker and benefit from a specific treatment (29).

Tumour grade is based on the degree of differentiation of the tumour tissue as assessed in histological examination. Currently, the widely recommended method internationally to determine breast cancer grade is the Nottingham (Elston-Ellis) modification of the Scarff-Bloom-Richardson grading system, also known as the Nottingham Grading System (NGS), which takes into account the following characteristics of breast tissue: tubule formation as a form of gland differentiation, nuclear pleomorphism and mitotic counts (28, 30). An overall grade of 1 to 3 is assigned, with 1 reflecting well-differentiated tumour, 2 for moderately-

differentiated tumour and 3 for poorly differentiated tumour. Higher grade or less differentiated tumours have been linked to more aggressive breast cancer and poorer prognosis (31, 32), and therefore histological grade is regarded as an important prognostic factor in breast cancer (30).

Assays of hormonal receptors expressed by breast cancer tissue, i.e. estrogen (ER) and progesterone receptors (PR), have also been useful in identifying breast cancer patients with different clinical profiles, and more importantly, in predicting clinical benefit from endocrine therapy (33). Patients with ER-positive breast tumours, which constitute the majority of invasive breast cancers (~70%), have shown better response to endocrine therapy compared to those with ER-negative status (34, 35). On the other hand, the prognostic relevance of ER and PR is still being debated due to a possible confounding by administration of endocrine therapy in early breast cancer (36). However, there is evidence that more favourable survival is seen with ER-positive compared to ER-negative breast cancers in patients who did not undergo endocrine treatment (36, 37). Although PR alone is only weakly associated with prognosis, better survivals have been reported in systemically untreated patients with both ER-positive and PR-positive cancers compared to those with negative expressions of both receptors (38).

Another important tumour marker in breast cancer is the human epidermal growth factor receptor 2 (*HER2* or *cERBB2*), a member of the epidermal growth factor receptor family which regulates cell proliferation, growth, and apoptosis (39). *HER2* gene amplification or overexpression of its transmembrane protein are found in 15-20% human breast cancers (40, 41), and have been linked to worse clinical outcome and recurrence (42–44). HER2 positivity is also a predictor of response to treatment with monoclonal antibody targeting the receptor such as trastuzumab (45). In addition, together with ER and PR, HER2 is used to identify patients with 'triple-negative' breast cancers, which are lacking expressions of ER, PR and HER2 and comprises approximately 15% of all breast cancer patients (46). Triple-negative breast cancers have been consistently linked to poor survival (47, 48), mostly attributed to the lack of receptors, i.e. ER and HER2 as therapeutic targets. At the moment, most triple-negative breast cancer patients in need of systemic therapy are treated with conventional chemotherapy where clinical outcomes are unsatisfactory (49), which prompts the need to establish new molecular target to improve survival in these patients.

The availability of high-throughput genomic and transcriptomic data has enhanced the identification of novel molecular markers of breast cancer during the last decade. One of the

highlights of this new phase in breast cancer research has been the classification of invasive breast cancers into at least four major molecular subtypes: luminal A, luminal B, HER2-enriched, and basal-like breast cancer (50–53) with more recent work suggesting as many as ten major subtypes (54). This finding complements the traditional clinical and pathological classifications of breast cancer and has reinforced the notion of breast cancer as a highly heterogenous disease (51) with further subtype-specific characterisation needed to allow more personalised treatment and improved clinical outcomes. In comparing molecular subtypes with traditional histopathological classes, a relatively large degree of overlap exists between luminal and ER-positive tumours, between basal-like and triple-negative cancers, and between HER2-enriched and HER2-positive tumours (53). Additionally, by using proliferation markers such as Ki-67, luminal-like breast cancers may be further divided into luminal A-like, which is usually HER2-negative with low PR and Ki-67 expression, and luminal B-like, which has high Ki-67 and may be HER2-negative or -positive (55). Differences in prognosis based on the original four molecular subtypes have been reported (50, 56), and assays utilising gene expression signatures to estimate prognosis and treatment outcomes such as PAM50, OncotypeDx and MammaPrint are now commercially available (52, 57–59). Despite these research advances, the widespread clinical deployment of genomic and transcriptomic tools to subtype breast cancer is hampered by the relatively high costs and resources needed. For this reason, the traditional immunohistochemical subtypes with their relatively well-defined clinical outcomes are still considered adequate in many clinical scenarios (60).

1.1.2. Epidemiology of breast cancer

1.1.2.1. Incidence, mortality, and survival

Due to influences of genetic and environmental exposures, the rates of breast cancer occurrence, deaths and survivorship vary in different races and geographical regions (61). This thesis utilises data from population-based cohorts in Sweden, so that the sections below discuss the epidemiology of breast cancer in both the UK and Sweden.

Breast cancer incidence, mortality, and survival in the UK

Female breast cancer is by far the most common cancer in the UK (62), accounting for about 30% of all cancer incidence and 50,285 new cases in females in 2011. More cases of female breast cancer have been diagnosed yearly over the past decades in the Great Britain, as shown by a 72% increase in European age-standardised incidence rates between 1975-1977 and 2009-2011 (Figure 3). Incidence rates only steadily increased (1-2% annually) between the mid-1970s and late 1980s. Higher increments observed during the 1980-1990 transition period was suggested to be caused by the introduction of national screening programmes (63), and the rates have relatively stabilised ever since. Similar trends from 1993 onwards (when data became available) are observed in the UK (64). Incidence rates are highest in older women, supporting a link with hormonal status. It was estimated that by the age of 50, around 10,000 women are diagnosed with breast cancer in the UK, whereas 80% of all breast cancer diagnoses are in women over 50s, and 24% in those aged 75 and over (64).

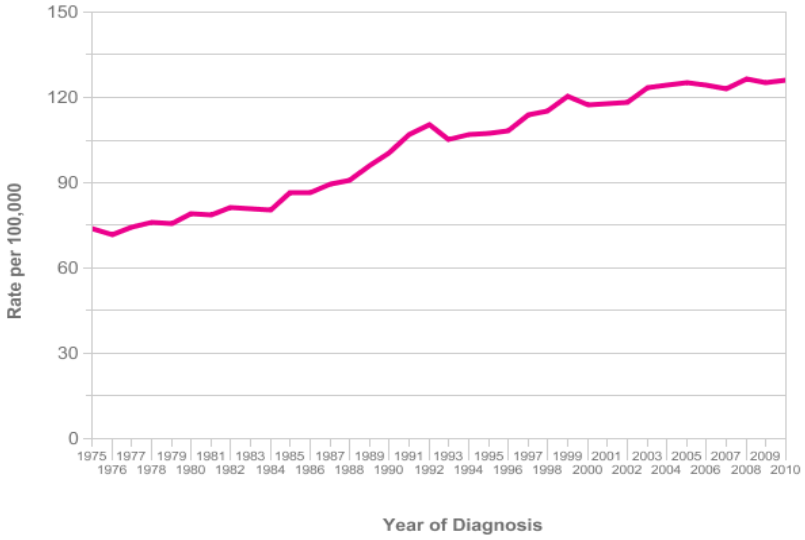


Figure 3. European age-standardised incidence rates of female breast cancer per 100,000 population, Great Britain, 1975-2011 (64).

Despite the advancement of its detection and treatments, breast cancer remains a leading cause of death in the UK, with 11,643 deaths in females in 2012 (64). A reduction in mortality rates by 36% have been observed between 1971-1973 and 2010-2012 (Figure 4), although an increase from the early 1970s until the mid-1980s was seen before the rates continued to decline. The highest mortality rates are observed in older populations, with an average of 46% breast cancer deaths in women aged 75 and older during the period 2010 to 2012 (64).

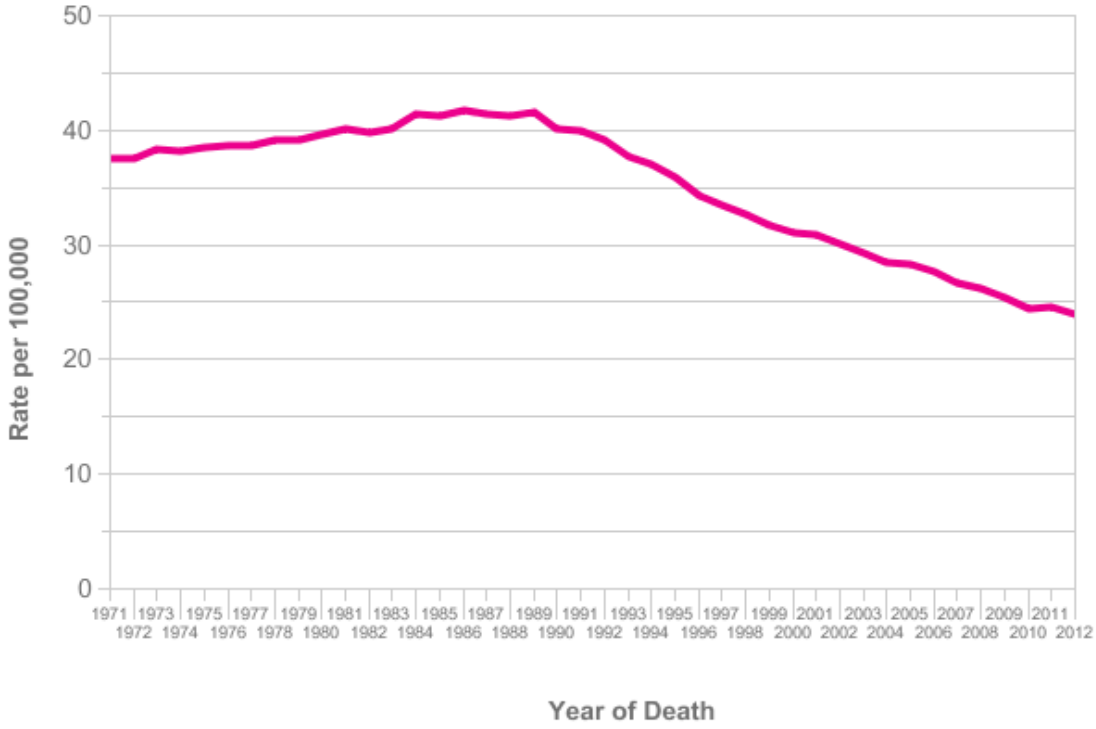


Figure 4. European age-standardised mortality rates of female breast cancer per 100,000 population, UK, 1971-2012 (64).

The UK has seen improvements in survival of breast cancer during the past decades. A 40% 5-year survival was observed during 1971-1972 in England and Wales, and this figure was predicted to reach 78% during 2010-2011 (64). As shown in Figure 5, the greatest gains in survival were seen in the 1990s, followed by continued but smaller annual increments throughout the 2000s. Some factors thought to contribute to the improved survival prior to the 2000s are mammography screening, which resulted in less advanced disease at diagnosis, innovations in breast cancer therapy such as adjuvant chemotherapy and tamoxifen, while improvement in later years is considered to result from health reforms directed towards better-quality management of breast cancer (65).

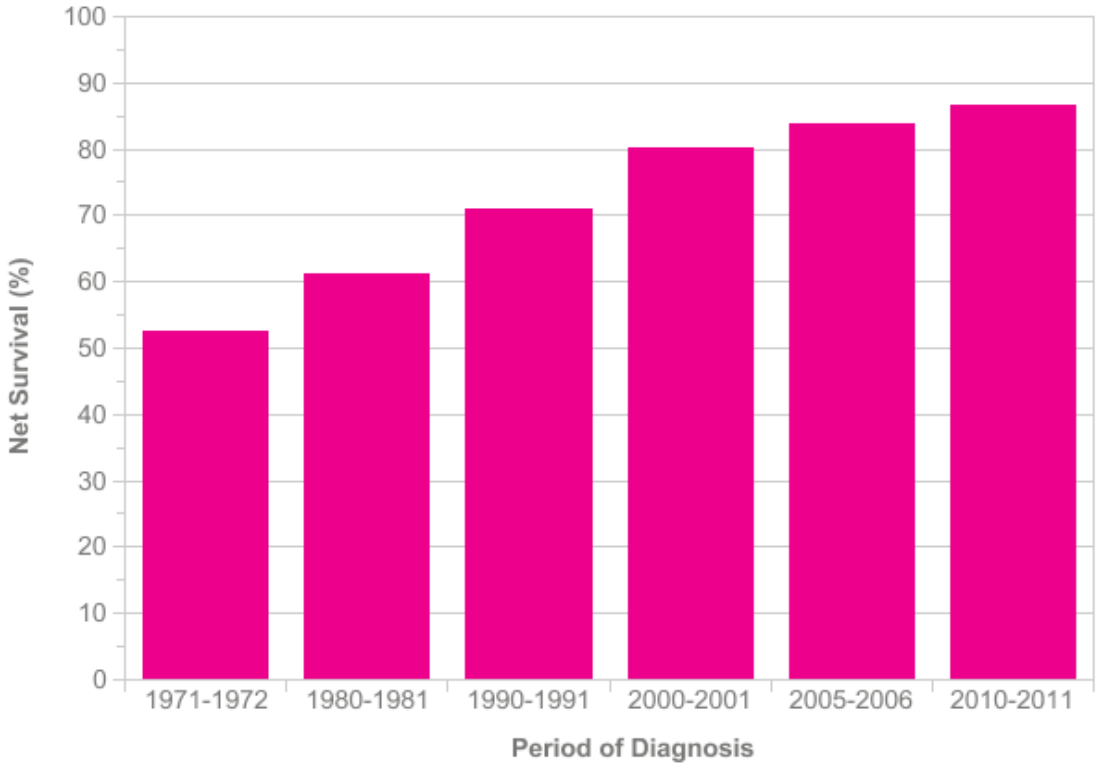


Figure 5. Age-standardised 5-year net survival of women with breast cancer aged 15-99, England and Wales, 1971-2011 (64)

Breast cancer incidence, mortality and survival in Sweden

Breast cancer is the most frequently diagnosed cancer in women in Sweden, with 8,382 new cancer cases or 30.3% among all cancers in females in 2011 (66). Increasing annual incidence rates of female breast cancer incidence are presented in Figure 6. Relatively weaker increments during the recent twenty years are seen compared to early 1990s, which may also be attributed to screening programmes. More than 80% breast cancer cases were diagnosed in women aged 50 and above (66), further signifying the importance of age in breast cancer incidence.

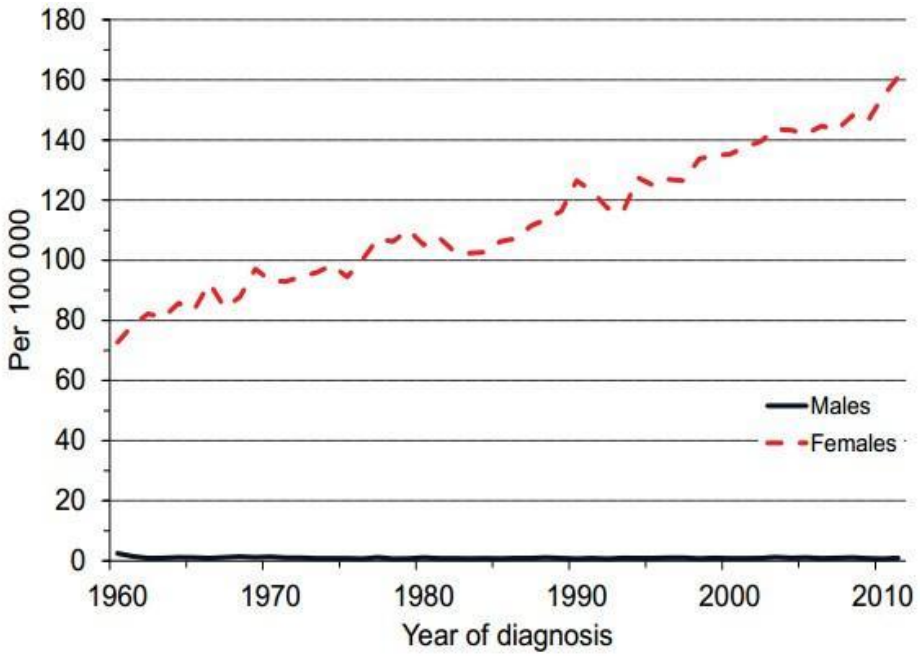


Figure 6. Age-standardised incidence rates of female and male breast cancer, Sweden, 1960-2010 (66).

As one of the most common causes of death in women, breast cancer contributed to approximately 42.8 deaths per 100 000 women aged 40 and over in Sweden in 2009 (67). From 1972 to 2009 (Figure 7), a yearly decrease of 0.98% in breast cancer mortality rates is observed in women aged 40 years and older. This trend was only modestly affected by the start of screening program both nationwide and in county-specific observations (68), which results in a relatively consistent decline over the years.

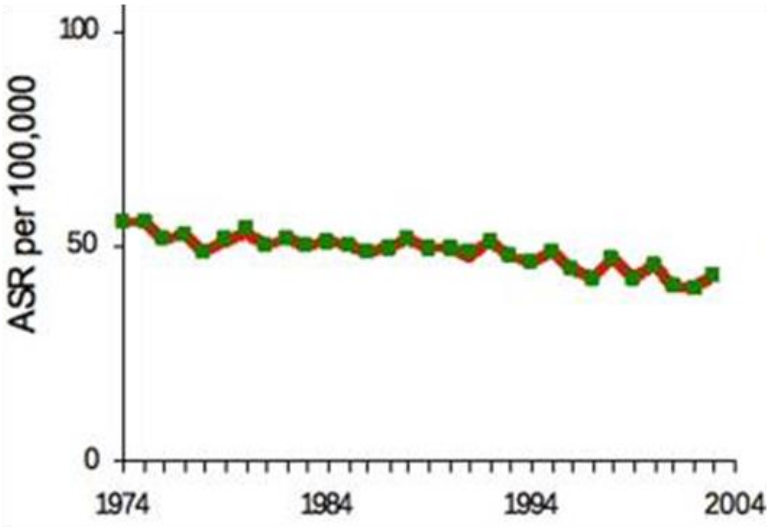


Figure 7. Age-standardised mortality rates breast cancer in females aged 40-79 in Sweden, 1974-2004. Adapted from Haukka and colleagues (69).

The relative 5-year survival of breast cancer patients in Sweden has increased from 64% in the 1960s to an estimated 88% during 2009-2013 (70, 71). As depicted in Figure 8, the greatest improvement in breast cancer survival occurred in the late 1970s. Advancement in treatment or national policies in breast cancer management are unlikely explanations to these early trends (72). Possible underlying factors include increased awareness of the advantage of breast cancer screening prior to the commencement of the screening programmes and the changing natural history of breast cancer and its determinants including lifestyle (70).

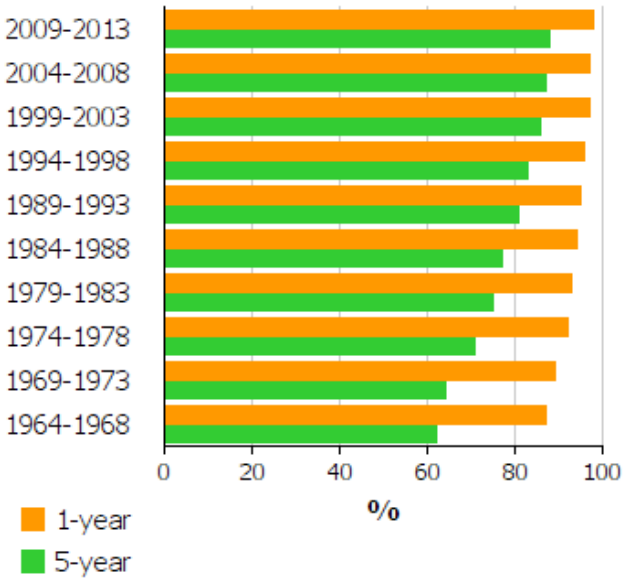


Figure 8. Age-standardised 5-year relative survival of women with breast cancer aged 0-89, Sweden, 1964-2013 (71)

Summary

Overall, breast cancer cases are still increasingly being diagnosed in both the UK and Sweden, whereas deaths from the disease decline over the years, and this trend is consistent with other European countries (2, 68). Screening programmes played an important part in the incidence and mortality trends. Following the start of national screening programmes in the late 1980s, a peak in diagnosis rates are seen in the UK and Sweden. However, a steep decrease in mortality rates following this time point was only seen in the UK. Mortality trends in Sweden are similar before and after the introduction of screening (68).

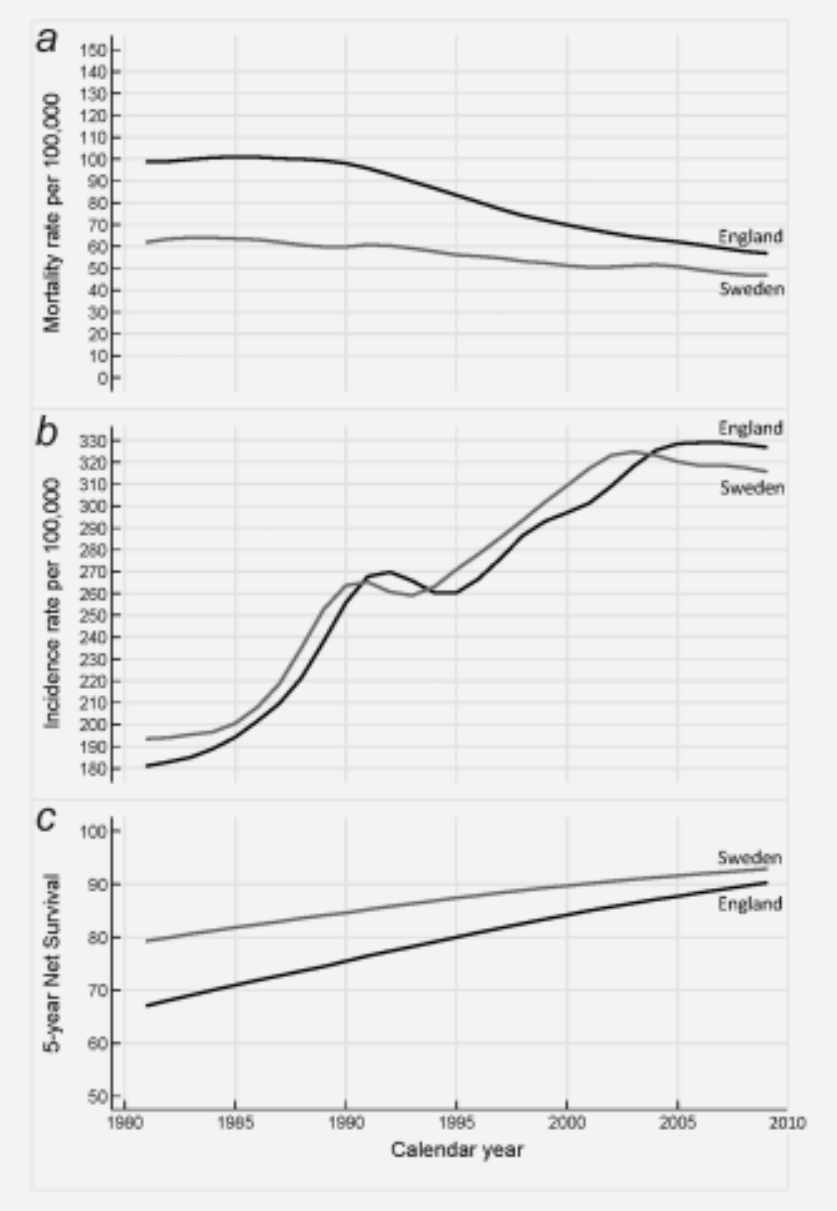


Figure 9. Annual mortality (a) and incidence rates (b) per 100,000 population and 5-year net survival (%) (c): breast cancer, women diagnosed aged 50–69 years in England and Sweden during 1981–2009 (73).

With regards to breast cancer survival estimates, there is apparent discrepancy between the two countries. Although marked improvement have been seen in England since the mid-1990s, survival remains lower compared to comparable developed countries including Sweden (6). Rather than data quality and changes in classification, these patterns are relevant to later diagnosis or differences in treatment, indicating quality in breast cancer management as the main determinant (74). Improvement in survival in Sweden tends to be less prominent in comparison to England especially after the 1990s (73), which is consistent to the comparable incidence rates and discrepancy in mortality rates between the two countries (Figure 9). The plateau-like trends observed for mortality and survival estimates in Sweden may be caused by a ceiling effect due to

high quality of breast cancer care in the country since the beginning of observation periods (65). Despite the steady narrowing of breast cancer survival disparity between the two countries and internationally, continuous surveillance is needed to ensure ongoing progress.

1.1.2.2. Breast cancer risk factors

Although it is estimated that 1 among 8 women will develop breast cancer in their lifetime, about 70% of them have no identifiable risk factors (75). Besides older age, a number of factors have been associated with higher risks of getting diagnosed with invasive breast cancer. Among the strongest risk factors are family history, inherited genetic mutations, previous breast cancer, mammographically dense breast, LCIS and atypical hyperplasia, and radiation to the chest (76–78). A family history of breast cancer in one first-degree relative has been estimated to increase the risk of getting diagnosed with breast cancer by 1.8 times compared to a lack of family history (79), and this risk multiplies with more numbers of first-degree relative with breast cancer, particularly at younger age. Among inherited genetic mutations, which accounts for 5-10% of all breast cancers, the most important by far are mutations of tumour suppressor genes *BRCA1* and *BRCA2* (77). The two genes were identified as breast susceptibility genes through linkage analysis in the mid-1990, and are associated with breast cancer risk of around 57% for *BRCA1* and 49% for *BRCA2* mutation carriers at age 70 (80). However, mutations of BRCA1 or BRCA2 only account for 15-20% of familial breast cancers (81, 82), leading scientists to believe that intermediate- and low-penetrance genes may jointly explain the remaining proportion of genetic susceptibility of breast cancer (83–86). Investigation into such polygenic susceptibility has advanced dramatically owing to genome-wide association studies (GWAS) (87), which has increasingly recognised common low-penetrance alleles associated with breast cancer risk such as variants of telomerase reverse transcriptase (*TERT*) and ataxia telangiectasia mutated (*ATM*) genes (88). In a recent meta-analysis including 11 GWAS on over 120,000 women, 15 novel low-penetrance genes were further identified, which altogether explained a further ~2% of breast cancer risk (89). It was estimated that assuming all the susceptibility genes could be identified, the half of the population at highest risk based on genetic scores would account for 88% of all breast cancer cases (90). Additionally, interaction between genes and environmental risk factors in breast cancer has been shown to affect the risk of breast cancer (91, 92), pointing toward the role of epigenetic alterations (93).

Reproductive factors have also been consistently associated with breast cancer risk (75, 94), which underlines the role of hormones. Higher risk of breast cancer has been reported with an

earlier age of menarche, later age of menopause, nulliparity, and late age at first childbirth, all of which are relevant to the cumulative number of ovarian cycles (95–97). Correspondingly, altered levels of endogenous sex steroid hormones have been linked to breast cancer incidence. Higher circulating estrogen and its metabolites corresponded to increased breast cancer risk among postmenopausal women (94, 98), and this association differed by estrogen metabolic profile (99). Among premenopausal women, the role of androgens has been implied, with positive associations observed between breast cancer risk and levels of testosterone, androstenedione, and dehydroepiandrosterone sulphate (DHEAS) but not sex hormone binding globulin (SHBG) (100). On the contrary, a reduced risk of breast cancer was associated to higher progesterone levels in the same study, For circulating prolactin, a positive association with breast cancer has been found among postmenopausal women using hormone replacement therapy (101). The impact of exogenous estrogen is further shown by an increased risk of breast cancer associated with oral contraceptives and hormone replacement therapy *per se* (102–105). On the other hand, a reduced risk of breast cancer has been observed following breastfeeding, and this association is independent of estrogen-driven risk factors including age at menarche, menopausal status, parity, and age at first childbirth (106).

Breast cancer susceptibility has been increasingly linked to lifestyle-related factors (107). Alcohol consumption has been repeatedly shown to correspond to a moderate increase in breast cancer risk, with an estimated 7-10 % risk increase for each additional 10g per day intake of alcohol (107, 108). Smoking, on the contrary, is not associated with breast cancer susceptibility apart from its correlation to alcohol consumption (108). Obesity has also been linked to an increased risk factor of postmenopausal breast cancer, with a 5 kg/m^2 increase in body mass index (BMI) corresponds to around 10% higher risk of breast cancer (109). There is also evidence that the risk of breast cancer increases with dietary fat intake and a lack of physical exercise (110, 111), further indicating a potential role of lifestyle modification in breast cancer prevention strategies.

Demographic factors apart from age also contribute to breast cancer susceptibility. Compared to other ethnicities, breast cancer risk is higher in Ashkenazi Jewish populations, which is attributed to the high frequency of *BRCA1* and *BRCA* mutations (112). Higher breast cancer incidence rates have been observed in women with higher socioeconomic status (113). However, multiple indicators of socioeconomic status and their close correlations to other risk factors such as race and lifestyle indicate its complex relationship with breast cancer risk (114). Other factors which

have been linked to increased breast cancer risk include height, exposure of diethylstilbestrol (DES), and history of endometrium, ovary, or colon cancer (77, 115, 116).

1.1.2.3. Breast cancer screening

Screening for breast cancer is performed with mammography, clinical breast examination, breast self-examination, and MRI. Mammographic screening has been suggested to result in a 15-20% risk reduction of deaths from breast cancer during 7 to 12 years of follow-up (117, 118). However, it is also associated with an estimated 19% of cancers being overdiagnosed, i.e. diagnosis of cancers which would not become clinically apparent without screening (11, 119). Additionally, a cumulative risk of a false-positive result of 61% has been reported for a 40- or 50-year old woman undergoing 10 years of annual mammogram. Despite the lack of its impact on survival, false-positive may cause harm to the patients through unnecessary invasive procedures, additional costs and psychological stress (118).

Since 2009, the U.S. Preventive Services Task Force has recommended that annual screening mammography is only performed for women aged 50 to 74 years, because the harm of screening in women beyond these age range is considered to outweigh the benefit (120). The same recommendation has been made by the Canadian Task Force of Preventive Health Care, whereas the U.K. National Health Service Breast Screening Program is using a slightly different cut-offs of 47-73 years and 3-year screening intervals. The decision of screening for younger women should be made case by case, and take into account individual risk of developing breast cancer. Baseline risk assessment is therefore an integral part of screening, and a number of risk prediction models have been proposed to provide the information. The most widely used tool to date is the Gail model, which takes into account age at menarche, age at first birth, number of first-degree relatives with breast cancer, number of previous breast biopsies, and presence of atypical hyperplasia to recognise those at increased risk of developing breast cancer (121). Despite some limitations when being applied to a multiethnic population, this model has been integrated in several screening guidelines including those of the U.S. National Comprehensive Cancer Network (122). Nevertheless, it is of note that for women with known or suggestive genetic predisposition for breast cancer, a different screening approach is recommended which involves genetic testing and counselling (123).

1.1.2.4. Chemoprevention

Breast cancer risk reduction through pharmacologic intervention include blockade of ER using selective ER modulators such as tamoxifen, and aromatase inhibitors such as exemestane. The American Society of Clinical Oncology recommends that the use of these agents should be discussed as an option to reduce the risk of invasive breast cancer, specifically ER-positive breast cancer in women at increased risk of breast cancer aged 35 years and older (124). Identification of those at increased risk is based on genetic predisposition, 5-year breast cancer risk of 1.7% or higher as assessed by the Gail model, and prior radiotherapy to the chest at young age and history of LCIS (122). A risk reduction in ER-positive breast cancer of 31-67% has been reported following chemoprevention with tamoxifen compared to placebo, however, no impact on breast cancer mortality is observed in previous studies (124).

1.1.3. Management of breast cancer

The management of breast cancer has evolved dramatically during the past few decades. Whilst treatment modalities are beyond the scope of this thesis, the following section briefly reviews current principles of primary breast cancer management to allow understanding into various factors that contribute to breast cancer survival, which is one of the outcomes studied in the thesis.

1.1.3.1. Diagnosis

Diagnostic work-up for breast cancer is performed for all symptomatic patients, and for those suspected to have breast tumour through breast screening. A triple assessment approach is recommended by most European professional bodies (125), which includes clinical assessment, mammography and/or ultrasound imaging, and core biopsy and/or fine needle aspiration cytology. It is advised that these assessments are carried out at the same visit (126). Clinical examination includes bimanual palpation of the breast and locoregional lymph nodes and an assessment for distant metastasis. MRI is not routinely recommended except in particular cases such as inconclusive results from conventional imaging. Detailed personal medical history, laboratory examination including full blood count, liver and renal function test, and menopausal status assessment should also be performed. Pathological diagnosis is based on a core needle biopsy, preferably by ultrasound or stereotactic guidance, or if not possible, at least a fine needle aspiration indicating carcinoma. Assessment of distant metastasis as well as comprehensive laboratory examination are not routinely performed in early breast cancer due to their lack of benefit for the patients (125).

WHO classification and the tumour-node-metastases (TNM) staging system should be used in making final pathological diagnosis of breast cancer, and includes information on tumour size, regional lymph node status, and distant metastasis. In addition to tumour stage, other clinical parameters including age, ER expression and histological grade are used in baseline risks assessment using several scoring systems estimating breast cancer prognosis, such as Nottingham Prognostic Index (NPI), Adjuvant! Online, or PREDICT score (127–129). When applicable, risk assessment using gene expression profiling as mentioned earlier is also performed.

1.1.3.2. Surgery

Breast surgery

Surgery remains the backbone of treatment for operable breast cancer. During the past 30 years, breast-conservation surgery (BCS) such as wide local excision has become the treatment of choice particularly in more developed countries. In the current guidelines, the target of local recurrence rates after wide excision and radiotherapy is <0.5% per year, and should not exceed 10% overall. In some patients, mastectomy is still performed due to reasons such as locally advanced breast cancer, positive surgical margins after multiple resections, or patient choice (125). Locally advanced breast cancer, defined as inoperable breast cancer which has not spread to distant sites, usually includes large operable primary breast tumours (stage IIB, IIIA) and/or those involving the skin or chest wall and/or those with extensive lymphadenopathies (stage IIIB, IIIC) (130). Following mastectomy, immediate or delayed breast reconstruction is recommended to achieve acceptable cosmetics. In selected patients, primary systemic therapy may precede surgery in order to downsize the tumour. In the case of downsizing, the choice of treatment is based on the assessment of clinical stage after primary therapy (60).

Axillary lymph node staging

Regional lymph node status is an important prognostic indicator in primary breast cancer. In previous years, axillary clearance had been the treatment of choice, but it is associated with lymphoedema in the upper limb especially when combined with radiotherapy. Currently, sentinel lymph node biopsy (SLNB) rather than full nodal clearance is the standard of care for axillary staging (131). Conventional axillary clearance is only recommended following detection of macrometastatic spread in the sentinel node if no radiotherapy is planned (60). This is because data from recent clinical trials comparing axillary dissection and axillary radiotherapy following 1-

2 positive sentinel lymph nodes in T1-T2 primary breast cancer showed equally low axillary recurrence rates, although lower adverse effects are observed with radiotherapy (132). However, other clinical trials showed no survival benefit of axillary dissection compared to SLNB alone in similar groups of breast cancer patients with micro- or macrometastasis (133, 134), which indicates that further considerations are needed in treating axillary spread of the disease.

Radiotherapy

Postoperative radiotherapy is strongly recommended after BCS (125) and after mastectomy with tumour size 5 cm or greater, a positive macrometastatic sentinel lymph node but no axillary dissection, or 1-3 involved nodes and adverse pathology (60). A locoregional recurrence risk reduction of roughly two thirds to three quarters has been reported following mastectomy (135). Whole breast radiation therapy with or without boost is the current standard of practice, although consideration of partial breast irradiation may be made for selected patients based on age and tumour characteristics (122). It is common for radiotherapy to follow chemotherapy when the latter is indicated.

Systemic therapy

Primary systemic therapy may take place prior to surgery and is known as 'neoadjuvant' treatment, and may also follow surgery as 'adjuvant' treatment. Systemic adjuvant treatment should be considered based on predicted sensitivity to particular treatment methods and benefit from their use and individual risk of relapse (125).

Chemotherapy

Also known as cytotoxic therapy, chemotherapy utilises chemical agents targeting vital cellular processes with no specificity towards cancer cells. Consequently, this treatment is associated with toxicity of the drugs to otherwise healthy tissues (26). The 2015 St. Gallen consortium recommended that the decision to administer adjuvant chemotherapy should be based on the intrinsic type of breast cancer, with the selective help from genomic examinations when available (60). For instance, in luminal B-like cancers, while there is pronounced benefit from chemotherapy, it could be omitted in cases with low-scores on first-generation genomic tests such as Oncotype Dx. For triple-negative breast cancers, however, chemotherapy remains the main component of systemic therapy, with anthracycline and taxane-containing regimens as the recommended treatment (60). Recently, the Early Breast Cancer Trialists' Collaborative Group

(EBCTCG) reported that clinical outcomes in breast cancer patients treated by taxane- or anthracycline-based adjuvant chemotherapy only slightly vary by known prognostic factors including age, nodal status, rumour diameter or differentiation, ER status, or use of tamoxifen (136). Nevertheless, individual absolute benefit while considering side effects of chemotherapy for breast cancer subtypes need to be assessed in order to correctly evaluate the benefit from chemotherapy (125).

Endocrine therapy

Adjuvant endocrine therapy has been routinely administered to all patients with ER-positive tumours, irrespective of chemotherapy and/or targeted therapy. Tamoxifen is the treatment of choice in premenopausal patients, although a switch to letrozole, an aromatase inhibitor, may be beneficial after the patient becomes postmenopausal (125). An aromatase inhibitor is also the choice for postmenopausal breast cancer patients (126). In addition, endocrine therapy as a neoadjuvant treatment has been increasingly recommended in treating postmenopausal patients with endocrine-responsive diseases, especially luminal-like breast cancers (60).

Biological therapy

Therapy targeting biological constituents of cancer cells has been regarded important in addressing the heterogeneity of breast cancer as well as other types of cancer. The use of trastuzumab, a monoclonal antibody binding the HER2, was first approved in the early 2000s (44), and despite the rapid growth of targeted cancer therapies, it has remained the most widely used biological therapy in breast cancer to date. Targeting HER2 using trastuzumab is strongly recommended in patients with HER-positive breast cancers, and has been associated with improved disease-free and overall survival (45, 125). Besides its use in adjuvant settings, HER2-targeted therapy is also recommended as a first-line treatment in HER2-positive diseases, except in patients with tumours with detectable ER and PR, where endocrine therapy alone is deemed adequate (137).

1.2. Inflammation

1.2.1. Biological basis of inflammation

Inflammation is an immunological process elicited by immune effectors, usually in response to tissue injury or infection. As defined by Cornelius Celsus in the 1st century AD, there are four cardinal signs of inflammation: *color*, *dolor*, *rubor* and *tumor*, which in Latin mean heat, pain, redness and swelling. The fifth sign, *functio laesa* or decrease in function, was later added by Rudolph Virchow in 1858 (138). A set of cellular processes are known to underlie inflammation and these clinical signs. Following an invasion by a pathogen, antigen-presenting cells (APC) such as macrophages and dendritic cells (DCs) may recognise antigens expressed by the pathogen through pattern-recognition receptors (PRRs) and engulf the pathogen, a process known as phagocytosis (139). After APCs have degraded the pathogen, they secreted inflammatory mediators such as cytokines and chemokines, substances with abilities to affect the behaviour of and attract other cells with relevant receptors, respectively. Cytokines and chemokines released by activated APCs initiate the inflammation cascade which produces the aforementioned clinical signs. The primary role of inflammation is survival upon injury or infection, and therefore, components of innate immunity such as neutrophils and other inflammatory cells and proteins are recruited from the blood into the site of infection in order to eliminate the pathogen. In addition, APCs are transported by lymph to nearby lymphoid tissue, where they activate adaptive immune response. This triggered recruitment of adaptive immunity components including antibody and effector T cells to the site of inflammation. Vasodilation and increased vascular permeability which occur during inflammation result in the redness, heat and swelling, whist pain is caused by inflammatory mediators such as prostaglandins. Inflammation and phagocytosis may also be triggered by the activation of a group of plasma proteins known as complement. Activation of the complement leads to coating or opsonisation of the pathogen's surface by complement fragments, and this complex is recognised by macrophages, after which phagocytosis takes place (139).

As mentioned above, two major types of immune mechanisms are known: innate or cellular and adaptive or humoral. Innate immune mechanisms are the first line of defence against invading pathogens characterised by immediate but short-term and non-specific immune responses carried out by macrophages, neutrophils, eosinophils, basophils, inflammatory mediators, natural killer (NK) cells and the complement (140). Adaptive immune mechanisms principally comprise lymphocytes with specific reactivity towards antigens and require a lag time to activate, usually a few days. However, the immune responses generated are long-lasting, specific, and unlike innate

immunity, they retain 'memory', i.e. repeated infection by the same pathogen will generate more amplified responses. The two classes of lymphocytes, i.e. B cells and T cells, give rise to antibody responses upon stimulation to B-cell receptor (BCR) and cell-mediated immune responses following T-cell receptor (TCR) stimulation, respectively. Activated B cells secrete antibodies, which are prns called immunoglobulins (Ig). At least five classes of immunoglobulins have been identified: IgA, IgD, IgE, IgG, and IgM (140). Protection against invading pathogens is mostly carried out by IgG and IgM, whereas IgA is important in mucosal immunity, IgE in allergy and parasite infection, and IgD functions mainly as an antigen receptor. Activation of B-cells upon antigen recognition is assisted by a class of T-cells known as T helper (T_H), mostly of the T_H2 subclass. Another class of T cells, cytotoxic T-cells (CTL), eliminate pathogens through inducing apoptosis or cell death upon stimulation by another T helper, T_H1. T_H1 also stimulates phagocytosis by macrophages, and therefore the balance between T_H1 and T_H2 determines the dominant type of adaptive immune responses against the pathogen.

As seen in Figure 10, both innate and adaptive immunity are interrelated and may underlie inflammation. For instance, antigen presentation by macrophages stimulates naive T cells to become armed T cells which are specific for that antigen. When the same antigen is in contact with surface immunoglobulin in B cells, armed T helper cells will recognise the antigen and secrete molecules to activate the B cells, the latter of which subsequently proliferate and differentiate into specific antibodies (139). Complement proteins also mediate antibody-mediated responses; hence it is a part of both innate and adaptive immunity. Furthermore, mast cells, the main effector of antibody-mediated allergic responses, may also stimulate phagocytosis in bacterial infection, indicating a role in innate immunity (141). All these immune responses are largely regulated by cytokines such as interferons (IFN), tumour necrosis factor (TNF) and interleukin-1 (IL-1) (140).

Systemic inflammation may follow local inflammation, resulting in general clinical signs which are induced by circulating cytokines including fever and an acute-phase response, i.e. modification of proteins synthesised and secreted by the liver cells into the circulation. These proteins induced by cytokines are called the acute-phase proteins, some of which have been shown to be involved in immune responses against invading pathogens (139). When the infectious agents are eliminated, a resolution of inflammation takes place, with the purpose of restoring tissue function (138). This is indicated by a flow of monocytes which differentiate into macrophages, the latter will engulf the dead bacteria or apoptotic cellular debris. When the cause

of inflammation is not resolved, a chronic inflammation may develop, mostly localised to the site of infection. This may result in specific local inflammatory responses such as formation of granulomas or epithelial tissue remodelling.

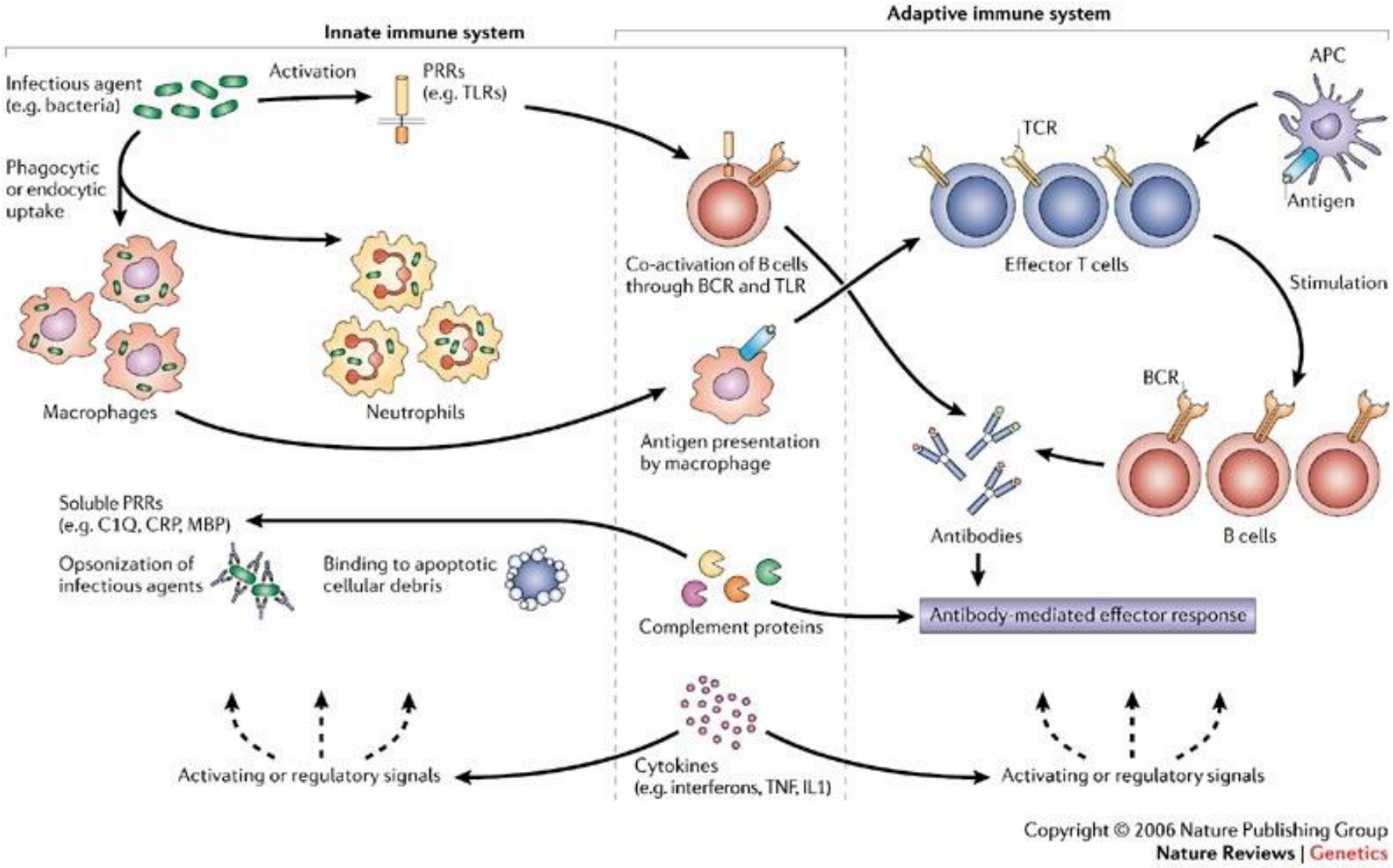


Figure 10. The interplay between innate and adaptive immune responses (142).

1.2.1.1. Inflammation, homeostasis and chronic diseases

During the past few decades, chronic inflammation has increasingly been described in conditions or diseases where the initiating trigger is unlikely to be an infection or wound, for instance obesity, diabetes, atherosclerosis, and allergy (138), the latter of which will be further discussed. An interesting observation is that a vicious cycle seems to take place, for instance inflammation may occur secondary to obesity, but chronic inflammation may also promote obesity-associated diabetes (143). A recent perspective of inflammation as a mechanism to preserve homeostasis has been proposed. Homeostasis reflects a complex machinery which maintains key regulated variables within an acceptable range, which may operate at the level of the entire organism, within tissue compartments, and within individual cells (144). For some variables such as body temperature, the aforementioned ranges or 'set points' are fixed at certain values, and any changes secondary to diseases will be immediately restored. However, in metabolic conditions such as obesity, continuous adjustments of set points occur to adapt with the cumulative changes inflicted by the environment, which in this case would be the increasing body weight and adiposity. Loss of homeostasis hence follows, inducing various tissue stressors such as endoplasmic reticulum stress, hypoxia and oxidative stress which are thought to evoke immune responses leading to inflammation (145). As a protective response, the resulting inflammation is engaged to defend and restore physiological functions by overriding perturbed homeostatic controls. Nevertheless, changes enforced by the inflammatory responses may be excessive or inappropriate, further worsening the conditions and leading to chronic pathological states.

1.2.1.2. Allergic inflammation

Deleterious effects of inflammatory responses are also observed in allergic disorders. The terms allergy and atopy have been used interchangeably in the literature to describe an abnormal adaptive immune response directed against non-infectious environmental substances or allergens such as pollen and food (146). However, atopy specifically refers to the predisposition to develop IgE-mediated immunological reactions to environmental allergens (147). In most cases, these reactions occur after the individual has become sensitised to the particular allergen, by producing specific IgE against it. On the other hand, allergic reactions, or traditionally known as 'hypersensitivity reactions', do not necessarily involve IgE, although a prior sensitisation against allergens may take place. Allergic inflammation, which occurs following an allergen exposure to sensitised subjects, may be classified into three temporal phases: early-phase, late-phase, and chronic (146). Early-phase reactions, also known as the type I hypersensitivity reactions (139), occur within minutes of allergen exposure and result from an immediate release of inflammatory

mediators by mast cells. In sensitised individuals, mast cells residing in tissues already have allergen-specific IgE bound to their surface high-affinity IgE receptors (FcεRI). Activation of mast cells occurs upon interaction with a multivalent allergen, leading to cross-linkage of IgE on their surface and degranulation or secretion of inflammatory mediators including histamine and cytokines such as TNF-α and IL-16 (141). Mast cells degranulation accounts for the local early-phase reaction, or a systemic one which is known as anaphylaxis. Some inflammatory mediators take longer time to be released and exerting their actions, and are responsible for late-phase reactions, which are usually observed 2-6 hours after allergen exposure, and often peak after 6-9 hours. In addition to delayed release of mast cells mediators, this late-phase reactions are also indicated to be attributed partly to allergen-stimulated T cells, eosinophils and monocytes (146). When a continuous or repeated allergen exposure occurs, inflammation may persist, invoking both innate and adaptive immune responses. As in asthma and chronic allergic rhinitis, this chronic allergic inflammation is associated with morphological and functional changes in affected organs such as epithelial tissue remodelling. It is, however, unclear how early- and late-phase reactions develop into chronic inflammation, and why some allergic individuals only develop early-phase reactions without experiencing the later phases (139).

1.2.1.3. Serum markers of inflammation

Systemic inflammation may follow local inflammation, and thus inflammatory-related proteins and cells may be detected in the circulation. These inflammatory markers have been useful in assessing inflammation-related conditions and diseases. Common clinical markers of inflammation were studied in this thesis. Serum leukocytes or white blood cells (WBCs) are routinely measured in the complete blood count and provide an estimation of their main components in the circulation: lymphocytes, neutrophils, eosinophils, basophils and monocytes, all of which are involved in innate and adaptive immunity (139). Also widely assessed as an indicator of inflammation are acute phase proteins, which are usually detected in the serum in abnormal amounts during inflammation. Among this group, C-reactive protein (CRP) is the most widely used marker in the context of both clinic and research. In healthy individuals, only minimal or no amount of CRP is detectable in the serum. A clinically raised level of CRP (10 g/L) indicates acute inflammation, whilst detectable but lower levels have been suggested to indicate low-grade inflammatory state (148). In addition to its role as a 'positive' indicator for inflammation, CRP is also known as a soluble PRR, which plays a role in innate immunity by opsonising invading pathogens and bind apoptotic debris (142). Haptoglobin, another acute-phase reactant, is also produced excessively by the liver during inflammation. Haptoglobin binds

haemoglobin released from red blood cells (RBC) during haemolysis, and therefore has been used as a marker for haemolytic anaemia in the clinic. However, a growing evidence shows that a specific haptoglobin phenotype, haptoglobin 2-2, exhibit antibody-like activity against bacteria, which suggests a role in immune responses (149). Some proteins are produced in lesser amounts by the liver during inflammation and are therefore known as 'negative' acute phase reactants. Among this category, albumin is the most frequently assessed, and its level has been shown to be inversely correlated to positive acute phase proteins such as CRP (150).

In addition to WBC and acute phase proteins, more recently established inflammatory markers have also been increasingly studied. Many studies directly measure systemic levels of cytokines such as interleukin-6 (IL-6), IL-8 and TNF-α, which have been investigated with respect to cancer (151–153). Another approach is to assess other pro-inflammatory markers which are regulated by these cytokines such as neopterin (154, 155). Nevertheless, measurements of these markers are less widely available compared to the conventional ones and they are not routinely performed in the clinic.

1.3. Inflammation and Breast Cancer

1.3.1. Linking inflammation and cancer

In 1863, Rudolf Virchow made the first connection between inflammation and cancer upon observing the infiltrates of leukocytes among neoplastic tissues (156). However, it was not until the last decade that scientists established the importance of inflammation in cancer. Grivennikov and colleagues summarised the link between inflammation and cancer to be multifaceted (157), in which inflammation may play a role in every stage of carcinogenesis, and reciprocal causations may take place. This is best shown in Figure 11, where a distinction can be drawn between inflammation that occurs prior to cancer and inflammation that follows cancer or its therapy. Whilst therapy-related inflammation is beyond the scope of this thesis, the two main extents of how inflammation is linked with cancer are further described below.

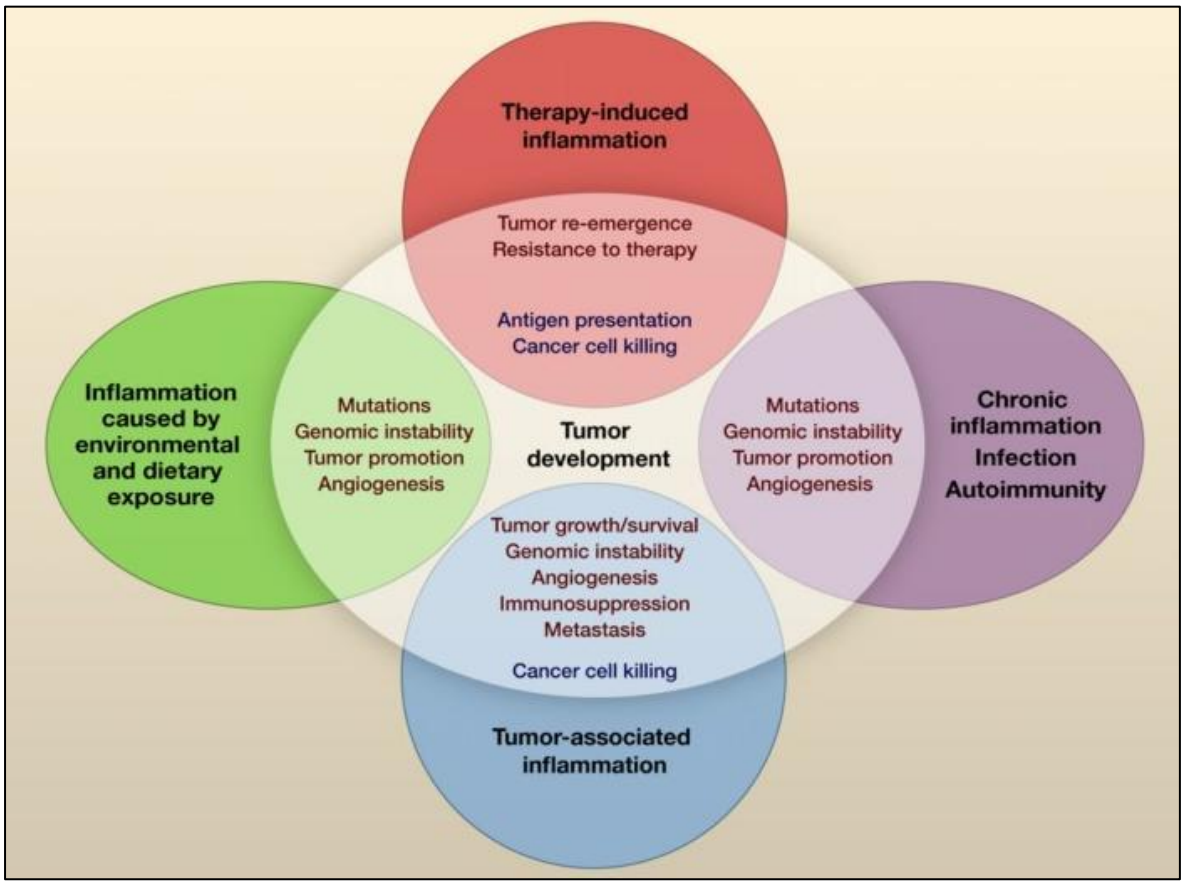


Figure 11. Types of inflammation in tumourigenesis and cancer (157)

1.3.1.1. Inflammation and carcinogenesis

The historical view of cancer can be summarised by the statement 'wounds that do not heal' (158), which clearly illustrates the role of inflammation, particularly the chronic type, in preceding cancer development. Inflammation is thought to be capable in triggering both tumour initiation and promotion. With respect to tumour initiation, inflammatory response may increase the formation of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI), which may induce mutation and genomic instability leading to malignant transformation (157, 159). However, it is uncertain whether ROS and RNI can initiate carcinogenesis independent of factors underlying inflammation, and it is thought that pro-inflammatory cytokines may enhance their intracellular tumour-initiating effects (157). In the context of tumour promotion, a number of signalling pathways linking chronic inflammation and cancer have been identified, including signal transducer and activator of transcription 3 (STAT3) and (nuclear factor kappa beta) NF-κB (160, 161). Pro-inflammatory cytokines may trigger the activation of STAT3 and NF-κB, which further activate genes responsible for cell survival, proliferation, and growth, as well as angiogenesis, invasiveness, motility, chemokine, and cytokine production, eventually leading to further inflammatory response and tumour promotion (162). These common pathways between inflammation and cancer may also imply that in some cancers, factors triggering inflammation are the real instigator of carcinogenesis, and that chronic inflammation may be a mediator rather than an independent promoter of cancer development. Correspondingly, STAT3 and NF-κB are also found to be activated by inflammation-related environmental and dietary exposures such as tobacco smoking, obesity, alcohol and infectious agents (161, 163), which further suggest the importance of underlying factors of inflammation in carcinogenesis.

1.3.1.2. Cancer-related inflammation

The two faces of immune responses in presence of cancer have been widely accepted: tumour-promoting and tumour-antagonizing. The first was described by Hanahan and Weinberg (13) as an 'enabling characteristic', which inadvertently supports cancer cells to thrive and disseminate. In healthy individuals, there is likely equilibrium between the primary function of immune system, which is to recognise and kill pathogens, with the secondary one: wound healing and immunosuppression. Nevertheless, the latter function is thought to predominate in cancer-related inflammation (164). As an evidence, instead of the conventional anti-pathogen neutrophils and M1 macrophages, alternatively-activated or polarised macrophages (M2) and neutrophils predominate tumour microenvironment (165). These inflammatory cells are directly recruited by tumour cells. Similar immunosuppressive activities in cancer have also been shown

with other immune effectors such as T-cells, B-cells, mast cells and DCs (157, 166). This evidence implied an involvement of both innate and adaptive immunity in tumour-related immunosuppression.

Tumour cells may also recruit myeloid-derived suppressor cells (MDSCs), a population of haemopoietic cells comprising immature precursors of DCs, granulocytes and monocytes/macrophages (167). MDSCs express specific markers such as CD11b and Gr1, and are suggested to being able to incapacitate T-cells. Additionally, MDSCs serve as a pool of cells replenishing polarised immune effectors in tumour microenvironment by cross-differentiating following stimulation by tumour-derived cytokines (168, 169). Recent evidence also showed that MDSCs promote mammary invasion by secreting cytokines that activates fibroblast migration, which emphasised their potential role in disease progression (170).

Despite abilities of a tumour to induce immune tolerance, anti-tumoural immune responses involving T-cells and NK cells have been reported in human cancers with a relevant significance in prognosis (171, 172). Nevertheless, cancer may directly counter anti-tumoural immune responses by secretion of inflammatory mediators such as transforming growth factor-β (TGF-β), which suppresses the activity of CTLs and NK cells (173). Moreover, cancer is a heterogeneous population of cells. Therefore, subpopulations of weakly immunogenic cells may survive and form solid tumours in spite of effective elimination of highly immunogenic cancer cells by the immune system. This process is known as immunoediting (174). Targeting the balance between the tumour-promoting and -antagonising effects of cancer-related inflammation may be a promising strategy to direct immune system toward eliminating cancer.

1.3.2. Inflammation and breast cancer

As in other solid tumours, inflammation may be involved in breast cancer promotion through the STAT3 and NF-$\varkappa$B pathways (175, 176). Similarly, leukocyte infiltrates have been suggested to play a role in breast cancer development and progression (177). The presence of B-cells has been reported in nearby lymph nodes and stroma of early breast cancer, as well as serum specific antibodies against tumour-associated antigens, which have been associated with worse prognosis (178). On the other hand, T-cells are more common in invasive breast cancer and have been linked to more favourable survivals (179, 180), implying a role of immune surveillance. Nevertheless, increased helper T cells and its increased ratio to CTLs are correlated to a poor prognosis in breast cancer (181), indicating tumour-associated immunosuppression. Similar

prognostic significance has been observed with other immune infiltrates such as macrophages and MDSC (181).

The role of systemic inflammation is subject of ongoing investigations in breast cancer. With regards to breast cancer prognosis, CRP levels at diagnosis or before treatment have been unequivocally shown to be associated with prognosis in a meta-analysis including ten studies, with a hazard ratio (HR) of 2.08 (95% confidence intervals [CI] 1.48-2.94) (182). Nevertheless, the role of CRP in the context of tumour promotion remains unclear. To present an overview of the association between serum CRP and breast cancer risk, a summary of epidemiological evidence linking prediagnostic CRP and incidence of breast cancer is shown in Table 1. Papers selected in this table were identified via PubMed literature searches conducted at the time this thesis was initiated and again in late August 2015. Searches were conducted using terms 'C-reactive protein', 'serum' and 'breast cancer' and restricted to human studies and English language publications published up to and including August 2015. References from selected papers were hand-searched to include additional papers.

Overall, the association between serum CRP and breast cancer risk remains conflicting, which aligns with results from a meta-analysis reporting a lack of association between baseline serum CRP and risk of breast cancer (183). Nevertheless, a recent case-cohort study showed an increased risk of breast cancer in postmenopausal women receiving hormonal therapy, but not those without (152), implying effect modification by hormonal factors. Although obesity has also been suggested to affect CRP levels (148), only one study showed adiposity status to modify the association between CRP and breast cancer risk (184). In the context of prognosis, there is evidence that higher postdiagnostic levels of CRP correspond to shorter progression-free and overall survivals of breast cancer patients (185). Given the correlation between CRP and other factors such as adiposity and external hormones (152, 184), it is essential to explore factors underlying inflammation to further delineate the association between inflammation and breast cancer.

Table 1. Overview of epidemiological studies assessing the link between serum CRP and breast cancer incidence

Publication	Study & Population	Design	Number of subjects, follow up	Exposure measurement	Risk of breast cancer	Notes
Il'yasova et al, 2005 (186)	The Health Aging and Body Composition Study; US; women aged 70-79 years	Cohort	1,305 without cancer; mean follow-up 5.5 years	CRP ELISA	HR: 1.32 (95% CI: 0.91-1.93) for every log CRP increase	Adjusted for age, race, and site
Siemes et al, 2006 (187)	The Rotterdam Study; Netherlands; women aged 55 years and over	Cohort	3,307 without cancer, mean follow-up 10.2 years	hs-CRP rate near-infrared particle immunoassay	HR: 1.23 (95% CI: 1.02-1.50) for every log CRP increase	Adjusted for age, smoking, body mass index, age at menarche and menopause, hormone use, and number of children
Zhang et al, 2007 (188)	Women's Health Study; US; female health professionals aged 45 and over	Cohort	27,919 without cancer, mean follow-up 10.1 years	CRP turbidimetry	HR: 1.02 (95% CI: 0.73-1.41) for highest vs lowest quintile; P_{trend} 0.81	Adjusted for age, intervention group, age at menarche, age at first pregnancy lasting ≥6 months, number of pregnancies lasting ≥6 months, menopausal status, age at menopause, family history of breast cancer, history of benign breast disease, postmenopausal hormone use, BMI, physical activity, multivitamin supplement use, smoking status, and alcohol intake
Allin et al, 2009 (189)	The Copenhagen City Heart Study, Denmark; women aged 20 and over	Cohort	5,369 without cancer; median follow-up 13 years	hs-CRP turbidimetry or nephelometry	HR: 0.9 (95% CI: 0.4-1.4) for highest vs lowest quintile; P_{trend} = 0.79	Adjusted for age at diagnosis, sex, cancer type, cancer stage, cancer histology, and time from blood sampling to diagnosis
Heikkila et al, 2009 (190)	The British Women's Heart and Health Study (BWHHS); UK; women aged 60-79 years	Cohort	3,274 without cancer	hs-CRP nephelometry	HR: 1.00 (95% CI: 0.76-1.31) for every log CRP increase; P_{trend} = 0.90	Adjusted for age, BMI, smoking, childhood and adult socioeconomic position, physical activity, HRT use, and NSAID use

Publication	Study & Population	Design	Number of subjects, follow up	Exposure measurement	Risk of breast cancer	Notes
Van Hemelrijck et al, 2011	The Apolipoprotein Mortality Risk (AMORIS) Study; Sweden; women aged 20 and over	Cohort	59,220 without cancer; mean follow-up 9.5 years	CRP turbidimetry	HR: 0.76 (95% CI: 0.41-1.43) for CRP > 50 mg/L vs < 10 mg/L; P_{trend} = 0.77	Adjusted for age, socioeconomic status, and history of circulatory disease
Prizment et al, 2013 (191)	The Atherosclerosis Risk in Communities (ARIC) study; US; women aged 45-64 years	Cohort	4,009 without cancer; 35,888 person-years	hs-CRP turbidimetry	HR: 1.27 (95% CI: 1.07-1.51) for every log CRP increase	Adjusted for age, center, education, BMI, waist, aspirin use, smoking status, hormone therapy use, menopausal status, age at menarche, and number of live births
Gaudet et al, 2013 (184)	The CPS-II Nutrition Cohort; US; women 50-74 years	Cohort	297 cases and 297 controls; follow-up NS	CRP ELISA	OR: 1.09 (95% CI: 0.70-1.70) for highest vs lowest tertile; P_{trend} = 0.16	Matched for age and race. Adjusted for time from last meal to blood draw, alcohol in the 24 hours before blood draw, prior diagnosis of diabetes, family history of breast cancer, and BMI
Gunter et al, 2015 (152)	The Women's Health Initiative Observational Study (WHI-OS); US; women aged 50-79 years	Nested case-cohort	875 cases and 839 subcohort	hs-CRP - nephelometry	HR: 1.24 (95% CI: 0.86-1.80) for highest vs lowest quartile; P_{trend} = 0.12	Adjusted for age, ethnicity, alcohol consumption, family history of breast cancer, parity, years of menstrual cycling, age at first child's birth, use of hormone therapy, endogenous estradiol levels (in non-hormone therapy users only), history of benign breast disease, body mass index, physical activity

Note: NS = Not Specified; OR = odds ratio; HR = hazard ratio; 95% CI: 95% confidence intervals

1.3.3. Underlying factors of inflammation and breast cancer

As mentioned before, inflammation occurs in an array of conditions such as altered metabolic states and allergy, which will be discussed further in this thesis. An understanding into how these underlying factors of inflammation may affect breast cancer is therefore important in gaining further insight into this complex association.

1.3.3.1. Allergy

Accruing evidence has indicated that allergy may be associated with the development of cancer, although findings in breast cancer are scarce. Hypotheses linking allergy and cancer are mainly divided into two groups which suggest that 1) allergy may reduce cancer risk and 2) it may increase cancer risk. The first includes the immunosurveillance hypothesis, which states that increased immune surveillance following hyperreactive immune responses may further hinder the development of cancer (192). Similarly, the prophylaxis hypothesis suggests that physical effects of allergic symptoms may prevent cancer, such as the act of sneezing in the attempt to remove allergens from the airway tract (193). The opposing hypotheses include a shift in T-helper balance which determines the type of immune responses elicited. Predominance of T_H2 over T_H1 underlies the hypersensitivity reactions in allergy, and is thought to divert immune responses from the tumour-eradicating T_H1 counterpart (194). Additionally, allergy is also a state of chronic inflammation, which may lead to initiation and promotion of cancer (158). Considering the complex nature of this relationship, it is important to first clarify how allergy is associated with overall cancer in order to gain insight into its potential role in breast cancer development.

Findings from observational studies studying allergy in relation to cancer risk remain hampered by the diverse methods used in assessing an individual to be 'allergic'. In general, two strategies have been employed: using self-reported history of allergic disorders, which is the most widely used method, and conducting objective measurements of allergy (195, 196). Besides its high feasibility and low costs, an advantage of using self-reported history is its ability to represent clinically relevant or symptomatic allergy. However, assessment of allergy relies on an individual's recall, and therefore may not correctly represent the state of allergy prior to cancer development. More importantly, different types of hypersensitivity reactions are underlain by different immunological mechanisms (139), which may be difficult to assess without any standardised objective assessment. Some studies utilising this method have shown a protective effect of allergy against cancer (197, 198), whereas associations with breast cancer remain unclear.

As atopy or IgE-mediated hypersensitivity reactions comprise the majority of allergy (146), measurements of total IgE levels in serum may be used as an objective assessment. However, IgE is also involved in other immune-related mechanisms such as the defence against parasite infections (139), indicating a lack of specificity when using total IgE. A preferable alternative is to specifically assess IgE sensitisation to a particular antigen through allergy tests such as skin prick test and serum allergen-specific IgE. The sensitivity and specificity of both tests are considered to be comparable (199, 200), but serum specific IgE is considered more feasible and safe due to a risk of anaphylaxis which follows skin prick test (201, 202).

Table 2 shows an overview of epidemiological evidence linking atopy, assessed with serum specific IgE, and cancer risk. Papers selected in this Table were identified via PubMed literature searches conducted at the time this thesis was initiated and again in late August 2015. Searches were conducted using terms 'immunoglobulin E', 'serum' and 'cancer' and restricted to human studies and English language publications published up to and including August 2015. References were hand-searched to include additional papers.

Overall, findings comparing cancer risk associated with atopic and non-atopic individuals vary by cancer types. The most consistent association has been observed for brain cancer, where atopic individuals are suggested to have a lower risk compared to non-atopic subjects (203–206). For breast cancer, a lack of association has been observed, which is similar to results from a meta-analysis assessing atopy diagnosed by skin prick test in relation to breast cancer (195), and findings using total serum IgE (196). Nevertheless, it should be noted that the skin prick test and specific serum IgE, despite their superiority to total IgE, only reflect IgE sensitisation, which is not always followed by clinical allergy (199, 200).

Recently, it has been suggested that the proportion of serum specific IgE among total IgE may better reflect clinical manifestation of atopy (207), but its importance with regards to cancer risk is yet to be reported. Additionally, all previous studies dichotomised results from specific IgE test into 'positive' and 'negative' in order to assign an individual to be atopic or non-atopic, and a loss of information may occur should there be a threshold of IgE levels which indicate specific immune responses associated with the susceptibility of developing cancer. Assessment of results from serum specific IgE assessment with respect to cancer risk in a dose-dependent manner, while taking into account serum total IgE, would therefore be a logical next step to explore this association.

Table 2. Overview of epidemiological studies assessing the link between serum specific IgE and cancer incidence

Publication	Study & Population	Design	Number of subjects, follow up	Exposure measurement	Outcome	Risk of cancer (95% confidence intervals)	Notes
Wang et al, 2006 (14)	The 'Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung' (ESTHER) , Germany, men and women aged 50-74	Case control	478 colorectal, 197 lung, 320 prostate and 381 female breast cancer cases and 4,271 controls	Serum specific IgE to inhalant allergens – UniCAP Phadiatop (positive, negative)	Prostate cancer Breast cancer Colorectal cancer Lung cancer	OR: 1.35 (1.00-1.83) for positive vs negative OR: 1.20 (0.87-1.66) OR: 1.29 (0.87-1.92) OR: 1.03 (0.80-1.34)	Adjusted for age, gender (except for prostate and breast cancer), education BMI, family history of cancer, smoking, alcohol
Ellison-Loschmann et al, 2007 (208)	The Spanish lymphoma case-control study	Case control	467 lymphoma and 544 controls	Serum specific IgE to inhalant allergens panels – Immulite 2000 (positive, negative)	Lymphoma	OR: 0.67 (0.45-1.00) for positive vs negative	Matched on age, sex, centre Adjusted for age, sex, centre, smoking, treated asthma or eczema
Melbye et al, 2007 (209)	The Scandinavian Lymphoma Etiology (SCALE) Case-Control Study, Denmark and Sweden, aged 18-74	Case control	2670 NHL cases and 2088 controls	Serum specific IgE to inhalant allergens – Phadiatop (positive, negative)	NHL	OR: 0.68 (0.58-0.80) for positive vs negative	Matched on age, sex, country Adjusted for age, sex, country, birth order, education, outdoor occupation
	The Finnish Maternity Cohort, women	Nested case control	198 NHL cases and 594 controls			OR: 0.74 (0.48-1.15)	Matched on age, length of storage
Petridou et al, 2007 (210)	Greece, women	Case control	103 female breast cancer cases, 103 controls	Serum specific IgE – HY-TEC (strong positive, positive, negative)	Breast cancer	OR: 1.73 (0.95-3.14)	Matched on age Adjusted for age, education, height, BMI, age at menarche, parity, age at menopause, alcohol

Publication	Study & Population	Design	Number of subjects, follow up	Exposure measurement	Outcome	Risk of cancer (95% confidence intervals)	Notes
Calboli et al, 2011(205)	The Physicians' Health Study (PHS), the Nurses' Health Study (NHS), the Women's Health Study (WHS), and the Health Professionals Follow-Up Study (HPFS), U.S., men and women aged 30+	Nested case control	181 glioma cases and 542 controls	Serum specific IgE – ImmunoCAP (positive, negative): Inhalant allergens Food allergen	Glioma	OR: 1.12 (0.77-1.62) OR: 1.03 (0.54-1.98)	Matched on year of birth, cohort, sex, intervention (in the PHS and WHS)
Schlehofer et al, 2011 (204)	The European Prospective Investigation into Cancer and Nutrition (EPIC), Europe, men and women aged 35-70	Nested case control	696 brain tumour cases and 1188 controls	Serum specific IgE to inhalant allergens – ImmunoCAP (positive, negative)	Glioma Meningioma Schwannoma	OR: 0.53 (0.30-0.95) in women OR: 0.89 (0.55-1.45) in men OR: 1.15 (0.65-2.02) in women OR: 0.64 (0.26-1.54) in men OR: 0.80 (0.32-1.99)	Matched on centre, sex, date of birth, age, data of sampling, time of sampling, length of follow-up Adjusted for education, smoking
Schwartzbaum et al, 2012 (203)	The Janus Serum Bank project, Norway, men and women, aged 20-49	Nested case control	594 glioma cases and 1177 controls	Serum specific IgE to inhalant allergens – ImmunoCAP (positive, negative)	Glioma	OR: 0.46 (0.23-0.93) for positive vs negative in women OR: 1.02 (0.72-1.44) for positive vs negative in men	Matched on age interval, sex, sampling date
Nieters et al, 2014 (211)	The European Prospective Investigation into Cancer and Nutrition (EPIC), Europe, men and women aged 35-70	Nested case control	1,021 lymphoid malignancy cases and 1,021 controls	Serum specific IgE to inhalant allergens – ImmunoCAP 100 (positive, negative)	Lymphoid malignancies	OR: 0.98 (0.77-1.23) for positive vs negative	Matched on centre, sex, blood donor status, age, date and time of sampling Adjusted for education, smoking
Olson et al, 2014 (212)	The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), men and women	Nested case control	283 pancreatic cancer cases and 544 controls	Serum specific IgE – ImmunoCAP (elevated, high, normal): Food allergen Inhalant allergens	Pancreatic cancer	OR: 2.83 (1.29-6.20) for positive vs negative IgE to food allergen in age 65+; OR < 1 in age < 65 No association with inhalant allergens	Matching on age, gender, race, calendar date of blood draw Adjusted for smoking, alcohol, education

Publication	Study & Population	Design	Number of subjects, follow up	Exposure measurement	Outcome	Risk of cancer (95% confidence intervals)	Notes
Skaaby et al, 2014 (213)	Denmark; men and women	Cohort	14,849 without cancer; median follow-up 11.8 years	Serum specific IgE to Inhalant allergens (positive, negative)	All cancer Specific site: Head and neck Colorectal Lung & bronchus Breast Prostate Urinary Melanoma Skin, other	HR: 1.00 (0.89-1.12) for positive vs negative HR: 1.74 (0.98-3.09) HR: 0.92 (0.64-1.32) HR: 0.78 (0.54-1.13) HR: 1.00 (0.73-1.37) HR: 0.79 (0.53-1.18) HR: 1.08 (0.60-1.96) HR: 0.95 (0.54-1.66) HR: 1.20 (0.98-1.47)	Age as timescale Adjusted for sex, study population, education, physical activity, smoking habits, alcohol intake, BMI

Note: HR = hazard ratio; OR = odds ratio

1.3.3.2. Metabolic disorders

There has been an emerging interest in how metabolic processes may be implicated in cancer progression. Two views have been generally accepted: cancer itself may manipulate cellular metabolism in its favour, and secondly, systemic metabolic states may also affect the clinical course of the disease. Using examples from these two perspectives, the following section discusses two common metabolic alterations which have been associated with both inflammation and breast cancer.

The Warburg effect

Among various metabolic abnormalities accompanying the development of cancer, one of the most important is altered cellular metabolism of energy and glucose (13). In normal cells, when oxygen is available, pyruvate generated during the breakdown of glucose is utilised to produce energy through oxidative phosphorylation. In contrast, tumour cells prefer pyruvate metabolism via anaerobic pathway regardless of oxygen availability, leading to inefficient fuel production and formation of lactate. This anomalous metabolic preference is called the Warburg effect or 'aerobic glycolysis' (214, 215). Aerobic glycolysis is also known to be closely related to inflammation. Activation of macrophages and T-helper cells leads to a metabolic shift to aerobic glycolysis (216), and correspondingly, a negative feedback of inflammation is provided by glycolytic products (217). In cancer cells, a positive regulation of aerobic glycolysis by pro-inflammatory cytokines such as TNF-α has also been documented (218, 219). However, it is suggested that chronic hypoxia secondary to tumour growth activates a feed-forward stimulatory loop between hypoxia pathways and aerobic glycolysis (220, 221), resulting in persistently altered metabolic states. Additionally, hypoxia induces endoplasmic reticulum stress in growing tumours (222, 223). As endoplasmic reticulum stress leads to accumulation of oxidative stress and activation of pro-inflammatory responses (224), it follows that a synergistic activation of aerobic glycolysis and inflammation may take place in malignancy, which may impact cancer progression.

A widely known marker for aerobic glycolysis is lactate dehydrogenase (LDH), which is the enzyme responsible for the conversion of pyruvate to lactate during glycolysis (225). LDH A and B subunits, coded by two different genes *LDH-A* and *LDH-B*, combine to construct five isoenzymes (LDH1 to LDH5) with selective distribution among tissues and in serum (226). The use of protein and gene expression of LDH has provided further insight into the role of aerobic glycolysis in cancer. For instance, in breast cancer, higher expression of LDH-A is observed in HER2-positive compared to HER2-negative cells, which indicates that the extent of glycolysis

varies across different subtypes (227). Moreover, an effect on breast cancer therapy has been indicated, as targeting LDH-A in combination with trastuzumab is able to inhibit trastuzumab-resistant breast cancer in experimental models (228). In the context of prognosis, a role for circulating levels of LDH has been suggested. Serum LDH is one of the five risk factors included in the International Prognostic Index (IPI) in diffuse large B-cell lymphoma (DLBCL) (229, 230), and a similar association is suggested in other cancer types such as lung (231–233) and haematological malignancies (234, 235). One study reported an association with breast cancer, where lower overall survival followed higher LDH levels among patients with bone metastases (HR: 4.50, 95% CI: 2.27-8.94) (236). Nevertheless, studies investigating the role of LDH in cancer survival were mostly performed in clinical trial settings where only patients with advanced disease were included, thereby limiting the generalisation of the current findings. Additionally, these studies focused on overall rather than cancer-specific survival, as reflected in a recent meta-analysis on LDH and survival in solid tumours where no studies reported cancer-specific survival as an outcome (237). Moreover, marked publication bias has been noted in this review. As serum LDH is also known to be increased in tissue injury, inflammation, haemolysis and myocardial infarction (238–240), it is necessary to confirm any association between serum LDH and cancer-specific death, and to assess whether such an association exists for breast cancer.

Altered glucose and lipid metabolisms

Disorders in glucose and lipid metabolism have been suggested as a mechanism linking obesity and breast cancer development (241, 242). In addition to their roles in carcinogenesis, increasing evidence suggests that abnormal levels of serum glucose and lipids impact survival in breast cancer patients (243–245). The mechanism through which glucose and lipid metabolisms may affect cancer progression is not clear, although a role of chronic inflammation is likely to play part. For instance, in obese individuals, increased adiposity is associated with increased production of cytokines including TNF-α, IL-6, and IL-1 (246). Apart from their correlation with obesity, abnormal levels of circulating glucose and lipids may also induce systemic inflammation, which may impact patient survival (185). Elevated serum glucose is thought to promote chronic inflammation through the generation of advanced glycation end products (AGEs), a product of non-enzymatic glycation of free amino group of proteins, lipids, or amino acids (247, 248). Similarly, serum lipids may contribute to inflammation through generation of lipid peroxides (249, 250). Furthermore, elevated levels of low-density lipoprotein cholesterol (LDL-C) as well as reduced levels of high-density lipoprotein cholesterol (HDL-C) have been linked to an increased activity of pro-inflammatory markers such as TNF-α and IL-6 (251, 252).

Both serum glucose and lipids are also linked to insulin resistance, which is signified by a compensatory production of insulin (253, 254) accompanied by an increase production of insulin-like growth factor I (IGF-I) (255). Insulin and IGF-I are known as strong growth factors (255), and have been implicated in breast cancer metastasis (256, 257). Additionally, insulin and IGF-I stimulate estradiol and testosterone production (258) further promote inflammation (259). All this evidence denotes the intricate network involving metabolic pathways and inflammation which may be important in breast cancer progression.

Studies linking serum glucose and lipids with breast cancer survival have yielded inconclusive results (243–245). A possible cause of this unclear link is the strong association between levels of glucose and lipid components such as triglycerides and total cholesterol with cardiovascular disease, which is the most common cause of death in the general population (260, 261). This common relationship may be explained by shared metabolic pathways including chronic inflammation and insulin resistance, both of which may drive formation of atherosclerotic plaque in addition to their aforementioned effects on cancer cells (242, 262, 263). Such risk correlations may thus result in a competing risk situation (264), where individuals with similar sets of risk factors are at risk of dying from both breast cancer and cardiovascular disease. To date, there is limited evidence on how competing risks affect the association between systemic glucose and lipid metabolisms and breast cancer survival. More investigations in this subject are thus needed to determine the true association between metabolic determinants and breast cancer progression.

1.4. Aims and Objectives

Breast cancer is a complex health problem which necessitates a multidimensional approach in order to reduce the burden it imposes. Several points formed the basis of research conducted within this thesis. First, despite a dramatic expansion of breast cancer characterisation in the last few decades, there is still a lack of understanding in the aetiology of most breast cancer cases, especially in patients without well-known genetic susceptibility. Secondly, although lifestyle has increasingly been linked to cancer and may provide opportunities for primary prevention, precise mechanisms leading to breast cancer development and progression remain unclear. Finally, current literature provides little evidence on whether there is any impact of other co-morbidities when assessing specific markers in relation to breast cancer survival. With longer life expectancy that follows advancement in medical treatment, there is an urge to study the complexity of survival determinants beyond cancer diagnosis in which other diseases are taken into consideration.

To address some of the aforementioned concerns, this thesis sought to investigate whether inflammation and associated underlying disorders are implicated in breast cancer development and progression. More specifically, this thesis had the following objectives:

1. To study the role of inflammation in the development of breast cancer by assessing serum CRP, albumin, haptoglobin and WBC in relation to breast cancer risk, severity at diagnosis and survival.

2. To evaluate any association between atopy and cancer risk using serum specific IgE against inhalant allergens and serum total IgE, with an emphasis on breast cancer.

3. To investigate the impact of prediagnostic serum lactate dehydrogenase on overall and cancer-specific survival following diagnosis and identify whether such associations differ with respect to tumour characteristics in breast cancer.

4. To assess the link between prediagnostic serum glucose, triglycerides, total cholesterol and breast cancer survival whilst accounting for competing outcomes.

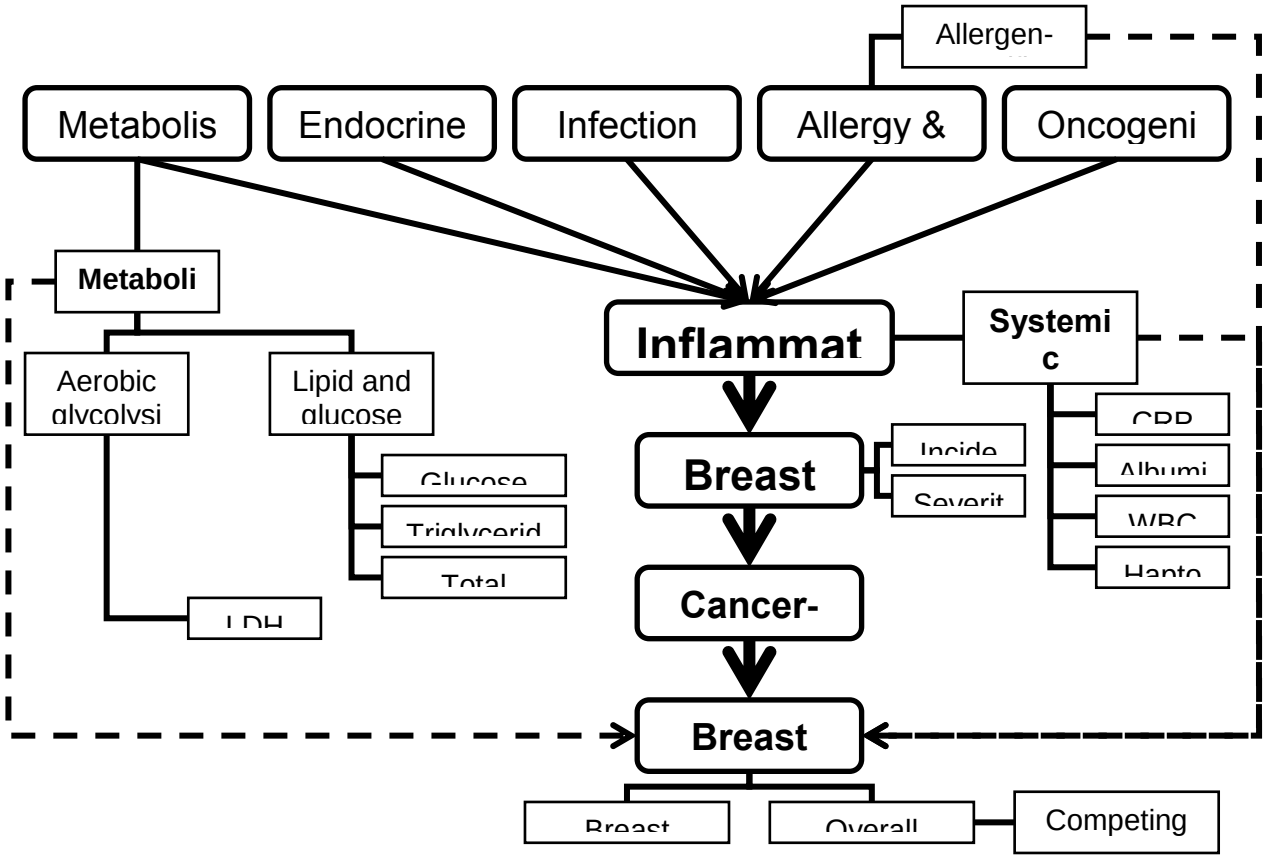


Figure 12. Schematic representation of research questions

Figure 12 graphically summarises the research questions posed in this thesis with relation to the plausible pathways linking inflammation, relevant clinical markers, and breast cancer. Through more understanding into the role of inflammation, its clinical markers and underlying factors in breast cancer, this thesis provides support for further mechanistic and clinical research utilising inflammatory markers in predicting breast cancer outcomes. Eventually, such findings are expected to contribute to shed light to the aetiology of breast cancer and its implications on disease outcomes, which may be further explored to design better intervention strategies.

Chapter 2: Methods

To allow clear description of research methods undertaken within the scope of this thesis, this section firstly explains the population in which analyses within this thesis were conducted and the source where data was obtained from. This is followed by explanations of specific methods to address each research question individually.

2.1. The Apolipoprotein Mortality Risk Study (AMORIS)

AMORIS is a large Swedish database containing 812,073 Swedish men and women, ranging in age from < 20 to 80 years-old and over. This cohort is based on a linkage between data from laboratory examinations performed in the Central Automation Laboratory (CALAB) in Sweden and information recorded in various Swedish National Registers using a 10-digit personal identifier number which is unique to every Swedish resident (265). As a result, AMORIS is currently one of the largest prospective cohorts containing information on serum biomarkers, cancer diagnosis, co-morbidities, vital status, socioeconomic status, and emigration (Please see Appendix 1.1. for an overview of published studies from The AMORIS cohort). This study complied with the Declaration of Helsinki and was approved by the Ethics Review Board of the Karolinska Institute. To understand the scope of AMORIS in this thesis, the linkages of the different data sources used in this cohort as well as the information available within each of them are further explained below.

2.1.1. Central Automation Laboratory (CALAB) database

CALAB was a major laboratory in Stockholm, Sweden, that served more than 3,000 physicians in the Swedish healthcare system. This laboratory was acknowledged for Good Laboratory Practice and internationally accredited in clinical chemistry, hematology, immunology, and microbiology (266). Participants of the AMORIS Study comprised Swedish residents whose blood samples were collected and examined in the CALAB between 1985 and 1996, and were mostly (67%) residents of the Stockholm County at the time of sample collection. These individuals were either healthy and had laboratory tests as a part of general check-up, or were outpatients referred for laboratory tests. None of the participants were inpatients at the time the samples were analysed. No clinical data were included in the CALAB database apart from information on blood test results and date of examination, participant age, sex, date of birth, and

personal identifier number, the latter of which was used to link the database to a number of Swedish National Registers in AMORIS.

2.1.2. Swedish National Registers

A personal identifier number, which has been established since 1947, allows data linkage amongst different national registers in Sweden (267). Swedish register linkages are mainly handled by two government agencies: the National Board of Health and Welfare and Statistics Sweden. The National Board of Health and Welfare is a government agency under the Ministry of Health and Social Affairs which is responsible for maintenance of health data registers and official statistics (268). This agency has a very wide range of activities and many different duties within the fields of social services, health and medical services, environmental health, communicable disease prevention and epidemiology. In addition to data maintenance, the agency also collects, compiles, analyses and passes on information related to the above fields, as well as developing standards based on legislation and the information collected. The second agency, Statistics Sweden, is an administrative agency with the main task of supplying both public and private customers with statistics for decision making, debate and research (269). This agency also supports and coordinates the system used for official statistics and publishes the vital statistics of Sweden, which are updated daily regarding births, migrations, deaths, and marital status. Register maintenance and linkages in health care often simultaneously involve both agencies, and have enabled feasible and efficient evaluation of Swedish health care, as well as data utilisation for large-scale medical research. National registers linked within the scope of AMORIS are shown in **Figure 13** and further described in the following sections.

Database	Coverage	N	Outcome	Demography	Lifestyle	Comorbidity
Clinical Cancer Quality Register	1997-2012	Breast: 14,934 – Prostate: 17,141 – Colorectal: 6,358	•			
Swedish Cancer Register	1958-2011	148,364	•			
Cause of Death Register	1961-2011	153,800	•			•
National Patient Register	1964/87-2011	Outpatient: 683,747 – Inpatient: 696,825	•			•
Total Population Register	1968-2012	800,587	•	•		
Census Data	1970-1990	783,922		•		
LISA database	1992-2010	799,647		•		
Medical Birth Register	1973-2011	204,449			•	
Multigeneration Register	1932-2011	812,073			•	
National Diabetes Register	1996-2011	58,985			•	•
Prescribed Drug Register	2005-2012	681,299			•	•
Karolinska Institutet (5 Research Cohorts)	1963-1990	29,000		•	•	•
AMORIS cohort (Biomarker measurements)	1985-1996	812, 073				

Figure 13. Swedish registers and databases linked to AMORIS

2.1.2.1. National Cancer Register

The Swedish Cancer Register, maintained by the National Board of Health and Welfare, was founded in 1958 and covers the whole population (268). It is compulsory for every health care provider to report newly detected cancer cases to the registry. A report has to be sent for every cancer case diagnosed at clinical, morphological, and other laboratory examinations as well as cases diagnosed at autopsy. Three types of data are available: patient demographics (personal identification number, sex and place of residence), medical data (**site of tumour, date of diagnosis, histological type, tumour stage**), and follow-up data (date and cause of death and date of migration). Cancer sites are coded according to the international (English) version of the Seventh Revision of the International Classification of Diseases (ICD-7) (Table 3). For medical data, information on histological type was collected since 1993 and tumour stage since 2004. Since the mid-80's, there are six regional registries associated with the oncological centres in each medical region of Sweden where the registration, coding and major check-up and correction work is performed. This regionalization implies a close contact between the registry and the reporting physician, which in turn simplifies the task of correcting and checking the material. The regional registries send information about newly registered cases and correction concerning those previously reported to the National Cancer Register on an annual basis. Underreporting of cancer cases in the Register was estimated to be 3.7% based on a comparison against the population-based Hospital Discharge Register in 1998 (270), and was highest among elderly (70+ years). In a more recent investigation, a comparison between the 2009 National Palliative Care Register with data from the National Cancer Register from 1958 to 2009 showed that 12.5% cases of cancer death were not reported to the National Cancer Register (271). However, this figure only represented underreporting in patients who received end-of-life care, and was likely to be lacking generalisability to the Swedish population as a whole.

2.1.2.2. Cause of Death Register

Since 1953, the Cause of Death Register has been overseen by the National Board of Health and Welfare as a database containing the **time and cause of death** of Swedish residents. Information on all those died during one calendar year and were registered in Sweden at the time of death was collected, regardless of whether the death occurred in Sweden or abroad. The causes of death are coded centrally at Statistics Sweden according to the international (English) version of the Eighth, Ninth and Tenth Revision of the ICD (ICD-8, ICD-9 and ICD-10) (Table 3). In 1995, death certificates were obtained for 99.7% of deaths registered in the database (272).

Table 3. International classification of diseases (ICD) codes used in AMORIS to classify cancer site

Cancer site	ICD-7	ICD-8	ICD-9	ICD-10
All cancer	140-207	140-209	140-208	C00-C97
Breast	170	174	174	C50
Prostate	177	185	185	C61
Pulmonary (primary)	162	162	162	C33, C34
Colorectal	153, 154	153, 154.0, 154.1	153, 154.0, 154.1	C18-C20
Gastroesophageal	150, 151	150, 151	150, 151	C15-C16
Hepatobiliary (primary)	155	155, 156	155, 156	C22-C24
Pancreas	157	157	157	C25
Kidney	180	189.0, 189.1	189.0, 189.1	C64, C65
Bladder	181	188	188	C67
Gynecological	171-176	180-184	179-184	C51-C58
Head and neck	140-148, 160-161	140-149, 160-161	140-149, 160-161	C00-C14, C30-32
Melanoma	190	172	172	C43
NMSC	191	173	173	C44
Central nervous system (CNS)	193	191, 192	191, 192	C70-C72
Thyroid	194	193	193	C73
Haematological	200-207	200-209	200-208	C81-C96

NMSC = Nonmelanoma skin cancer

2.1.2.3. Hospital Discharge Register

The Hospital Discharge Register was established by the National Board of Health and Welfare as part of the National Patient Register in 1964 and has had complete national coverage since 1987 (273). This Register contains data on patients (personal identification number, sex, place of residence), hospital identification, administrative data (date of admission and discharge, acute/planned admission) and medical data (main and secondary discharge diagnoses and major interventions), which enable assessments of **history of other diseases or co-morbidities**. The above information is delivered to the Centre for Epidemiology (EpC) at the National Board of Health and Welfare from each of the 21 county councils in Sweden and is updated once a year (268). Every discharge corresponds to one record in the Register. In 2006, the completeness of discharge data was 99.4% for personal identifier number and 99% for the main diagnosis. A low number of drop-out has also been recorded, with an estimated rate of less than one percent in 2007.

2.1.2.4. Total Population Register

In 1968, Statistics Sweden established the Total Population Register, which contains information about the people that live in Sweden and about where in the country they reside (274). This Register is presently managed by the Swedish Tax Agency and contains information on names, personal identifier number, residence, birth place, immigration and **emigration** including

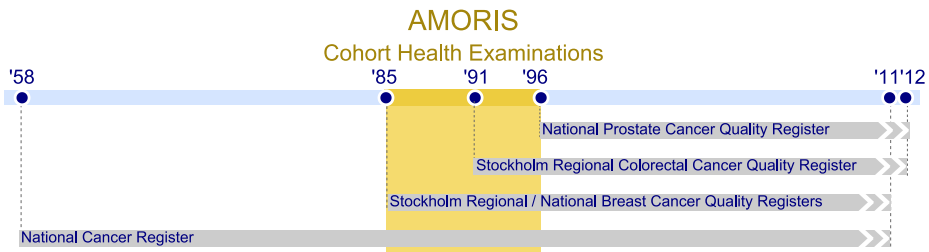
addresses abroad (275). If registered for the first time, a person will receive a personal identifier number which will be used in subsequent official administration in Sweden afterwards. From 1970 to 1990, the Total Population Register conducted a five-yearly Population and Housing Census, which collected information from questionnaires and nationwide registries including data on demographics, occupation and earnings. Information on occupation and earnings was used to create a variable for **socioeconomic status** which classifies gainfully employed subjects into manual and non-manual workers, designated as blue-collar and white-collar workers (266).

2.1.2.5. Multi-generation Register

The first census in Sweden took place in 1961 and was later used to serve as a basis for the Multi-generation Register which also utilises personal identifier number (274). This database contains parentage information including personal identifier number of an individual and their biological (and adoptive) parents, date and place of birth, **number of children**, and date of immigration. In 2005, information on mother was found in 97% and on father was found in 95% of total individuals born in Sweden that year. The equivalent frequencies of those born outside Sweden are 27% and 22%, respectively. Lower frequencies are present for mothers and fathers of those born during the 1930s, which align to the introduction of the personal identifier number in January 1, 1947. Therefore, information on biological mothers is available in 98% of individuals born in 1947 and 100% of those born in 1961 and onwards. The equivalent numbers for fathers are 94% for those born in 1947 and 98% for those born in 1961 and onwards.

2.1.2.6. Breast Cancer Clinical Quality Register

The Breast Cancer Clinical Quality Register contains detailed information regarding breast cancer tumour characteristics. In the AMORIS Study, a linkage in 2014 to the Stockholm Regional Breast Cancer Clinical Quality Register provided data from 1985 onwards on breast cancer tumour characteristics at diagnosis including tumour size, nodal status and distant metastasis which were used to classify tumours by **TNM stage** according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 7th edition. In some patients, further information on **grade**, **receptor status (ER, PR and HER2), and menopausal status** were available. The follow up of AMORIS participants relative to this quality register and the National Cancer Register is shown in Figure 14.



Cancer Register	1958-1984	1985-1996	1997-2001	2002-2011	2012
Breast	2,742	6,495	4,388	10,416	na
Prostate	412	4,006	4,125	14,970	na
Colorectal	1,050	4,775	3,955	10,552	na
All cancers	21,686	45,447	31,696	86,712	na
Quality Registers					
Breast	na	4,660	3,112	8,109	na
Prostate	na	138	3,252	13,588	163
Colorectal	na	628	1,687	3,762	58

Figure 14. Follow-up of cancer incidence based on national and regional registers

2.2. Data Collection and Analysis

Although all studies within this thesis were based on the AMORIS population, different methods were employed to address different questions as previously described. Therefore, the following section is divided into four parts, each corresponding to specific data collection and analytical techniques conducted for each research question.

2.2.1. Systemic inflammatory markers in relation to breast cancer risk, severity, and survival

2.2.1.1. Study population

This study investigated the associations of serum markers of inflammation with risk of breast cancer, disease severity, and survival following diagnosis (Please see Appendix 1.2. for publication of this study). We selected all women aged 20 and older with baseline serum CRP and albumin from the AMORIS population (N=155,179) (Figure 15), among which 6,606 developed breast cancer during follow-up. All participants with any prior history of cancer at baseline were excluded. To exclude reverse causation, only women with follow-up time of more than 2 years were deemed eligible. Among this population, 149,258 women who had at least 15 of any biomarkers at CALAB, which represented general health check-up, showed similar characteristics to the main study population. Therefore, analyses were conducted on the full study population (N=155,179) as described above.

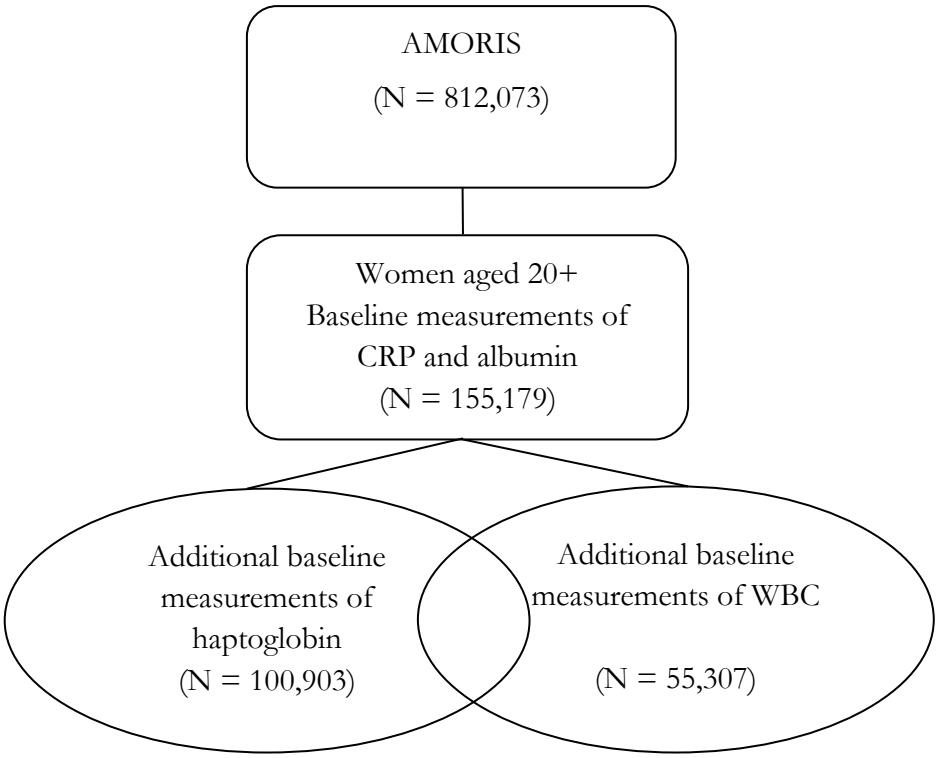


Figure 15. Overview of study population

2.2.1.2. Exposure and outcome assessments

Serum CRP (mg/L), albumin (g/L), haptoglobin (g/L) and WBC (10 x 10^9/L) were measured at baseline with standard laboratory methods as detailed in Table 4. High sensitive CRP (hsCRP) was not available at any time in the period of blood sample collection (1985-1996), so that CRP concentrations <10 mg/L could not be measured precisely (266). However, this cut-off point of 10 mg/L is widely accepted as the upper limit of the health-associated reference range (276) and was therefore used in this study. Levels of serum inflammatory markers were assessed as high or low based on their clinical cut-offs used in CALAB: CRP 10 mg/L, haptoglobin 1.4 g/L, and WBC 10 x 10^9/L. For albumin, a cut-off point of 40 g/L was used instead of 35 g/L due to the small number of participants with low albumin levels.

In addition to serum inflammatory markers, the following baseline information was obtained from AMORIS: socioeconomic status (white collar, blue collar, unemployed or unknown), parity (nulliparous, 1+ children), history of hospitalisation for liver disease, rheumatic disease, diabetes and lung disease (ever, never).

There were three outcomes investigated in this study: breast cancer diagnosis, breast cancer severity, and breast cancer survival. A severity classification which was a modified version of the conventional St. Gallen criteria was used (277), and breast cancer cases were categorised into three levels of severity: mild, moderate, and severe based on information on age at diagnosis, tumour stage, and ER status (Table 5). When investigating breast-cancer survival, both breast cancer-specific and all-cause death were assessed as the outcomes.

Table 4. Laboratory methods used to measure selected serum markers in CALAB

Marker	Instrument	Method	Total imprecision
CRP	AutoChemist® PRISMA (New Clinicon, Bromma, Sweden) 1985-1992 and Technicon DAX™ 96 Multichannel Analyzer (Bayer Diagnostics, Tarrytown, USA) 1993-1996	Immunoturbidimetry using reagents from Orion Diagnostics (Helsinki, Finland)	≤12.0% CV
Albumin	AutoChemist® PRISMA (New Clinicon, Bromma, Sweden) 1985-1992 and Technicon DAX™ 96 Multichannel Analyzer (Bayer Diagnostics, Tarrytown, USA) 1993-1996	Bromcresol green method	≤2.0% CV
Haptoglobin	Hitachi 911 Automatic Analyzer (Boehringer Mannheim, Germany)	Immunoturbidimetry using reagents from Orion Diagnostics (Helsinki, Finland)	≤ 5.6% CV
WBC	Coulter® STKS Haematology System (Coulter Corporation, Hialeah, USA)	The STKS is a haematology flow cytometer that automatically performs blood cell counting from whole blood samples	<2.7% CV
Specific IgE	Pharmacia CAP® System (Thermo Fisher Scientific, formerly Pharmacia Diagnostics AB, Uppsala, Sweden)	Immunoassay	≈10.0% CV
Total IgE	Immunoassay System ES 700 (Boehringer-Mannheim, Germany)	Enzyme-linked immunosorbent assay (ELISA)	<5%
LDH	AutoChemist® PRISMA (New Clinicon, Bromma, Sweden) 1985-1992 and Technicon DAX™ 96 Multichannel Analyzer (Bayer Diagnostics, Tarrytown, USA) 1993-1996	Enzymatic spectrophotometry	<4% CV
Glucose	AutoChemist® PRISMA (New Clinicon, Bromma, Sweden) 1985-1992 and Technicon DAX™ 96 Multichannel Analyzer (Bayer Diagnostics, Tarrytown, USA) 1993-1996	Enzymatic method (GOD-PAP)	<2.2% CV
Triglycerides	AutoChemist® PRISMA (New Clinicon, Bromma, Sweden) 1985-1992 and Technicon DAX™ 96 Multichannel Analyzer (Bayer Diagnostics, Tarrytown, USA) 1993-1996	Enzymatic method (GPO-PAP)	≤5.0% CV
Total cholesterol	AutoChemist® PRISMA (New Clinicon, Bromma, Sweden) 1985-1992 and Technicon DAX™ 96 Multichannel Analyzer (Bayer Diagnostics, Tarrytown, USA) 1993-1996	Enzymatic method (CHOD-PAP)	≤2.7% CV

CV: Coefficient of variation

Table 5. Severity classification of breast cancer at diagnosis

Mild	Moderate	Severe
		< 40 years and TNM Stage II
ER+ or ER-	ER+ and age ≥ 40	OR
AND	AND	ER- and TNM Stage II
TNM Stage I	TNM Stage II	OR
		TNM Stage III or IV

2.2.1.3. Statistical analysis

First, risk of breast cancer based on different levels of baseline serum inflammatory markers was assessed. Follow-up time was defined as the time from baseline measurements until date of breast cancer diagnosis, death from any cause, emigration, or end of study, whichever occurred first. Hazard ratios and confidence intervals for incident breast cancer were obtained with Cox proportional hazards regression, comparing women with high to low levels of CRP, albumin, haptoglobin and WBC. The assumption of proportional hazards was met by assigning variables as time-varying covariates. All models were adjusted for age at baseline measurements, socioeconomic status, and parity in individual analyses and a fully adjusted model. In addition to being included as a continuous variable, age was also adjusted as categories (<30, 30-40, 40-50, 50-60, 60-70, 70 years and older). Additional adjustments were performed for diabetes and lung disease as a proxy for smoking. A sub-analysis based on menopausal status at baseline used age as a proxy of menopause. We conducted stratification analysis by menopausal status using two approaches. In addition to simple stratification analysis using age of 50 years as a proxy, we performed truncation analysis in which premenopausal women were followed to age 50 after which they were censored. In the assessment of postmenopausal risk, individuals with measurements before age 50 entered the study at age 50 by means of delayed entry. This approach including truncation analysis has previously been used in studies combining two populations in which reaching a certain age or menopause was a condition to enter the study (278). Since obesity is linked to inflammation and breast cancer (109, 148), analysis was repeated in the subgroup of women with baseline BMI while adjusting for BMI. Additionally, since disease of the liver may impair production of CRP, albumin and haptoglobin (279, 280), a sensitivity analysis excluding 521 women with history of liver disease was performed. A similar sensitivity analysis was performed by excluding 1,436 women with history of rheumatic disease since levels of CRP and haptoglobin may be influenced by the disease and its medications (281).

To assess the association between prediagnostic inflammatory markers and breast cancer severity, ordered logistic regression was used to estimate proportional odds ratios of more severe breast cancer by categories of CRP, albumin, haptoglobin and WBC. This analysis allowed the use of three severity categories as ordered outcomes and was performed in 5,108 breast cancer patients with available information on disease severity. The proportional odds assumption was met for all markers. The models were adjusted for age and menopausal status at diagnosis, period of diagnosis and interval time between baseline measurements and breast cancer diagnosis.

Finally, inflammatory markers were investigated in relation to all-cause and breast cancer-specific death in 6,606 women with breast cancer. Patients were followed up until death, emigration or end of study, whichever occurred first. Cox proportional hazards models were used, adjusting for age and menopausal status at diagnosis, tumour stage, ER status, period of diagnosis, and interval time between baseline measurements and breast cancer diagnosis. Missing variables were assigned as a different value for menopausal status (18%), tumour stage (18%) and ER status (32%). To further illustrate this association, cumulative incidence functions for all-cause and breast cancer-specific death were displayed by categories of CRP, albumin, haptoglobin and WBC. Gray's test for equality of cumulative incidence functions was performed to assess differences in cumulative risk of death with respect to baseline markers.

All analyses were conducted with Statistical Analysis Systems (SAS) release 9.4 (SAS Institute, Cary, NC) and R version 3.0.2 (R Foundation for Statistical Computing).

2.2.2. Atopy and cancer

2.2.2.1. Study population

To study the associations of atopy with cancer in general (Please see Appendix 1.3 and 1.4 for publication of this study), and more specifically with breast cancer, a total of 8,727 men and women aged 20 or above were selected among the AMORIS participants. All included participants had serum specific IgE test for inhalant allergens and total IgE measured in CALAB between 1992 and 1996 and no history of cancer at baseline measurements.

2.2.2.2. Exposure and outcome assessments

Serum levels of IgE against common inhalant allergens were measured using an immunoassay (Table 4). Results of allergen-specific IgE test were expressed as scores ranging from 0 to 6 which represent different levels of IgE from undetectable up to high concentrations of IgE (kU/L) as displayed in Table 6. Inhalant allergens tested in this study are presented in Table 7. Apart from the IgE scores, no information on continuous levels of specific IgE was available. As with previous studies, any scores higher than 0 (which correspond to specific IgE levels of ≥0.35 kU/L) were defined as IgE sensitisation and the presence of atopy (282). When multiple allergens were tested at baseline, results for all specific IgE measurements were collected. Atopy was defined as having at least one positive result among all the tested allergens. In addition to atopy status, highest specific IgE scores recorded at baseline examinations were considered as the exposure in this study.

Table 6. Specific IgE scores in CALAB and corresponding serum concentrations

Specific IgE score	Serum concentrations (kU/L)	Serum IgE levels
0	<0.35	Absent/undetectable
1	0.35 – 0.70	Low level
2	0.70 – 3.50	Moderate level
3	3.50 – 17.5	High level
4	17.5 – 50	Very high level
5	50 – 100	Very high level
6	≥100	Very high level

Table 7. Description of specific IgE against inhalant allergens tested in AMORIS

CAP code	Allergen-specific IgE
D1	House dust: Dermatophagoides pteronyssinus IgE
D2	House dust: Dermatophagoides farinae IgE
D3	Mites: Dermamophagoides microceras IgE
D70	Mites: Acarus siro IgE
D71	Mites: Lepidoglyphus destructor IgE
D72	Mites: Tyrophagus putresentiae IgE
D73	Mites: Glycyphagus domesticus IgE
D74	Mites: Euroglyphus maynei IgE
E1	Cat dander IgE
E3	Horse dander IgE
E4	Cow dander IgE
E5	Dog dander IgE
E6	Guinea pig epithelium IgE
E70	Goose feathers IgE
E78	Parrot (budgerigar) feathers IgE
E82	Rabbit epithelium IgE
E84	Hamster epithelium IgE
E85	Chicken feathers IgE
E86	Duck feathers IgE
E87	Rat IgE
E89	Turkey feathers IgE
G1	Grass pollen: Sweet vernal IgE
G12	Grass pollen: Cultivated rye EgE
G13	Grass pollen: Velvet grass IgE
G5	Grass pollen: Perennial rye-grass IgE
G6	Grass pollen: Timothy grass IgE
G7	Grass pollen: Common reed IgE
H1	House dust IgE
H2	House dust, Hollister-Stier Labs IgE
M1	Molds/yeast: Penicillium notatum IgE
M2	Molds/yeast: Cladosporium herbarum IgE
M3	Molds/yeast: Aspergillus fumigatus IgE
M6	Molds/yeast: Alternaria tenuis IgE
T12	Tree pollen: Willow IgE
T14	Tree pollen: Cottonwood IgE
T2	Tree pollen: Grey alder IgE
T3	Tree pollen: Common silver birch IgE
T4	Tree pollen: Hazel IgE
T7	Tree pollen: Oak IgE
T8	Tree pollen: Elm IgE
T9	Tree pollen: Olive IgE
Tx5	Tree pollen mix IgE
Tx9	Tree pollen mix IgE
W10	Weed/flower pollen: Lambs quarters, Goosefoot IgE
W12	Weed/flower pollen: Golden rod IgE
W19	Weed/flower pollen: Wall Pellitory (*P. Officinalis*) IgE
W20	Weed/flower pollen: Nettle IgE
W6	Weed/flower pollen: Mugwort IgE
W7	Weed/flower pollen: Marguerite, ox-eye daisy IgE
W8	Weed/flower pollen: Dandelion IgE
W9	Weed/flower pollen: English plantain, ribwort IgE

Besides serum specific IgE, measurements of total serum IgE (Table 3) were collected in 8,727 participants. Total IgE levels were categorised based on its clinical cut-off points into low (< 25 kU/L), moderate (25-100 kU/L) or high levels (≥100 kU/L) (196). Other information included in this study was age at baseline measurement (years), sex (male, female), socioeconomic status (white collar, blue collar, unemployed or unknown), and history of hospitalisation with chronic pulmonary disease including asthma (ever, never). Additionally, period of measurement was categorised (1992-1993, 1994-1995, 1996) to account for a long recruitment period.

Diagnosis of incident cancer was the main outcome of interest in this study. Apart from overall cancer, all cancers excluding nonmelanoma skin cancer (NMSC) were assessed given the suggested underreporting of NMSC in European countries (283). Furthermore, the ten major cancer sites in the study population were also evaluated: prostate, female breast, colorectal, gynaecological, haematological, melanoma, pulmonary, bladder, NMSC, central nervous system, and kidney cancer. The secondary outcomes of this study were all-cause and cancer-specific deaths.

2.2.2.3. Statistical analysis

Multivariable Cox regression was used to estimate hazard ratios (HR) with corresponding 95% confidence intervals (CI) of overall risk of cancer by atopy status (yes, no) in all participants. Follow-up time was defined as the time from baseline measurement until cancer diagnosis, death from any cause, emigration, or end of study, whichever occurred first. Additionally, the trend between specific IgE scores against inhalant allergens and risk of cancer was evaluated by assessing scores in groups (0, 1-2, 3-4, 5-6) as an ordinal scale. Levels of IgE are known to substantially decrease with age (284). Additionally, the assumption of hazard proportionality was not met when serum specific IgE was used as a time varying covariate. Therefore, all analyses were performed using age as the time scale.

Models were adjusted for sex, socioeconomic status, and period of measurement, history of chronic pulmonary disease to account for asthma and as a proxy for smoking given their association to IgE sensitivity and risk of lung cancer (147, 285). Analyses were repeated in men and women separately. A further adjustment for categories of total IgE levels was performed in the second model. To evaluate any effect modification by total IgE levels, analyses were stratified according to total IgE levels. Analysis was subsequently performed for all cancer excluding NMSC and the ten most common cancer sites with adjustment for total IgE levels. The analysis

was repeated in men and women, but only assessed the five most common cancer sites due to the small number of cases.

For the secondary objective, prediagnostic specific IgE was studied in relation to survival after cancer diagnosis. Three cancer patients were excluded in the analysis because the diagnosis of cancer occurred at time of death, leaving 686 individuals with cancer in the final analysis. Follow-up time was defined as the time from cancer diagnosis until death from any cause, emigration, or end of study, whichever occurred first. Kaplan-Meier curves were used to assess overall survival by atopy status and scores of specific IgE, and statistical differences were assessed with the log-rank test. Cox regression was used to quantify the risks of all-cause and cancer-specific deaths by IgE sensitisation status and allergen-specific IgE scores with age at diagnosis as time scale. The models were adjusted for the time interval between baseline IgE measurements and cancer diagnosis, and total IgE levels.

All analyses were conducted with Statistical Analysis Systems (SAS) release 9.4 (SAS Institute, Cary, NC) and R version 3.0.2 (R Foundation for Statistical Computing).

2.2.3. Serum lactate dehydrogenase and survival following cancer diagnosis

2.2.3.1. Study population

In this study (Please see Appendix 1.5 and 1.6 for publication of this study), the association between serum LDH and survival was studied in those diagnosed with cancer in AMORIS. A total of 7,895 men and women aged 20 and older was selected, with histopathological diagnosis of incident cancer between 1986 and 1999 and measurements of prediagnostic serum LDH. Follow-up time was defined as time from cancer diagnosis until the date of death from any cause, emigration, or end of study (31 December 2011), whichever occurred first.

2.2.3.2. Exposure and outcome assessments

Serum LDH (ukat/L) was measured automatically (Table 3). Prediagnostic LDH was defined as the last measurement taken within 3 years prior to cancer diagnosis. For a secondary analysis, LDH levels measured within six-month intervals prior to cancer diagnosis were collected and an average was calculated for persons with >1 measurement within any interval time. The standardised value (z-score) of LDH was calculated by subtracting with the mean and dividing by the standard deviation. Both non-transformed LDH and its z-score were normally distributed. Since LDH cut-offs vary across laboratories, its upper limit of normal (ULN) was used to categorise LDH into low and high levels (≤ ULN, > ULN).

Also included in the analysis were socioeconomic status (white collar, blue collar, unemployed or unknown) and Charlson co-morbidity index (CCI). CCI consists of 17 groups of diseases with a specific weight assigned to each disease category (286), and these weights were then summed to obtain an overall score, resulting in four co-morbidity levels (0, 1, 2, and 3+) indicating no co-morbidity to severe co-morbidity. Period of diagnosis was categorised (before 1989, 1989-1993, 1993-1997, 1997 onwards) to account for the long period of recruitment and differences in cancer management over time. Information on tumour stage was available for 877 breast cancer cases (Stage I to IV).

The outcomes of this study were overall death and cancer-specific death. For specific cancer sites, cancer-specific deaths were defined as individuals whose primary cause of death matched their primary cancer diagnosis.

2.2.3.3. Statistical analysis

Kaplan-Meier curves were used to assess overall survival by categories of prediagnostic LDH, and statistical differences were assessed with the log-rank test. Cox proportional hazard regression was used to estimate hazard ratios (HR) and their 95% confidence intervals (CI) of overall and cause-specific death by z-score and categories of LDH, adjusting for age at diagnosis. The proportionality of hazards assumption was met after assessing time-varying covariates which were the cross-products of each variable and time. The multivariable model was further adjusted for sex, socioeconomic status, CCI, and period of diagnosis. To assess serum LDH at time of diagnosis, analyses were repeated in a subgroup of 1,657 participants who had their baseline LDH measured within 3 months prior to cancer diagnosis.

To observe the association between baseline LDH and survival in specific cancers, a similar multivariable analysis was performed by major cancer sites. For breast cancer, there was information available on tumour stage, so that analyses were repeated while adjusting for tumour stage. Cumulative incidence functions were used to display cumulative risk of dying from all-cause and cancer, and statistical difference was assessed with Gray's test for equality of cumulative incidence functions. Kaplan-Meier curves and cumulative incidences were displayed only for deaths up to ten years after diagnosis since trends past this cut-off point were similar to the ones presented. However, statistical analyses were performed using data for the whole follow-up.

In a secondary analysis, the aim was to observe any temporal association between LDH and survival in cancer patients. The average of LDH was measured for each six-month time interval before cancer diagnosis and associations of LDH with overall and cancer-specific cancer survival for each lag time were examined. The models were adjusted for age at diagnosis, sex, socioeconomic status, CCI, period of diagnosis, and stratified by cancer sites. A subset analysis was performed for breast cancer, stratified by tumour stage (I-II, III-IV).

All analyses were conducted with Statistical Analysis Software (SAS) release 9.4 (SAS Institute, Cary, NC) and R version 3.0.2 (R Project for Statistical Computing, Vienna, Austria).

2.2.4. Associations of serum glucose and lipids with breast cancer death

2.2.4.1. Study population

The objective of this study was to examine the association of serum glucose and lipids with breast cancer death in presence of any competing risks (Please see Appendix 1.7 and 1.8 for publication of this study). From the AMORIS population, 1,798 women with an incident diagnosis of breast cancer between 1985 and 1999 who had baseline measurements of serum glucose, triglycerides and total cholesterol within three months to three years prior to diagnosis were selected. Follow-up time was defined as the time from diagnosis until death from any causes, emigration, or end of study (31 December 2011), whichever occurred first.

2.2.4.2. Exposure and outcome assessments

Serum levels of glucose (mmol/L), triglycerides (mmol/L), and total cholesterol (mmol/L) were measured with standard methods (Table 3). All three markers were measured at the same day, within three months to three years prior to diagnosis. This timeframe was selected to capture metabolic derangements during ongoing malignancy process while excluding effects of breast cancer diagnostic or treatment interventions. Triglycerides levels were not normally distributed, and therefore log-transformed values of all markers were used in addition to their quartiles in the analysis. Apart from serum markers, information was collected for fasting status (fasting, not fasting, unknown) and socioeconomic status (white collar, blue collar, unemployed or unknown).

The main outcome of the study was breast cancer-specific death. In order to evaluate the association between the studied markers and this outcome, death from cardiovascular disease and other causes was used as competing outcomes.

2.2.4.3. Statistical analysis

Analyses for this study were performed in two stages. First, multivariable Cox proportional hazards regression was used to assess the association between log-transformed values and quartiles of glucose, triglycerides and total cholesterol and the risk of breast cancer death as the primary outcome, cardiovascular death and other death as competing outcomes. Adjustment was performed for potential confounders including age at diagnosis, socioeconomic status, and fasting status at baseline measurements. Glucose, triglycerides and total cholesterol were each analysed while adjusting for the other two markers as continuous variables. The proportionality of hazards assumption was met after assessing time-varying covariates which were the cross-

products of each variable and time. To assess any potential competing risk, proportions of deaths from breast cancer, cardiovascular disease and other causes were displayed by quartiles of glucose, triglycerides, and total cholesterol.

Commonly used methods in survival analysis, including Cox' proportional hazards, rely on the assumption of non-informative censoring. When this assumption is met, any censoring due to non-primary events does not affect one's risk of developing the primary outcome, thus such a risk is proportional to the levels of risk factors or covariates observed. However, when competing risks are an issue, a heterogeneous association between covariates and the primary outcome may exist, reflecting subpopulations or classes with different mortality risk profiles. To address this potential heterogeneity, the association between serum glucose, triglycerides and total cholesterol and breast cancer survival was subsequently investigated using a latent class proportional hazards model. Latent class analysis has been used to identify different classes or latent variables within a given population which underlies the pattern of association between observed covariates (287). In medical research, the latent class variable has been incorporated into various regression analyses, including Cox proportional hazards models, to allow identification of subgroups with different risk profile (288–290). To capture heterogeneity in the context of breast cancer survival, an extension of the proportional hazards model was used, which encompassed latent class variables in addition to glucose, triglycerides and total cholesterol, assessed as continuous variables. The number of latent classes present in the cohort was identified with Bayesian model selection. To assess breast cancer-specific death whilst accounting for competing risks, breast cancer death was incorporated as the primary outcome and deaths from cardiovascular disease and other causes as non-primary outcomes into the latent class proportional hazards model. Class membership probabilities were retrospectively predicted based on associations between covariates and events. Independent samples T-test and Chi^2 test were used to assess differences in characteristics of study participants and predicted class membership. Latent class-specific cumulative mortality functions were further displayed for breast cancer, cardiovascular and other death by levels of the three markers. Finally, hazard ratios for breast cancer, cardiovascular and other death by levels of glucose, triglycerides, and total cholesterol were estimated for each latent class according to the maximum-a-posteriori (MAP) likelihood, which took into account all three outcomes (291).

Descriptive analysis and Cox proportional hazards model were performed with Statistical Analysis Software (SAS) release 9.3 (SAS Institute, Cary, NC) and R version 3.0.2 (R Project for

Statistical Computing, Vienna, Austria). Latent class proportional hazards model were performed with Advanced Survival Analysis software version 0.2.16 (A.C.C. Coolen, M. Rowley, M. Inoue, London, UK).

Chapter 3: Results

Relevant findings from studies within this thesis are reported in the following section. Similar to the previous chapter, results are presented for each research question separately.

3.1. Systemic Inflammatory Markers in Relation to Breast Cancer Risk, Severity, and Survival

Characteristics of study participants are shown in Table 8. During a mean follow-up of 18.3 years, 6,606 women were diagnosed with breast cancer, of whom 1,474 died, with breast cancer being the main cause of death in 736. Most of the study participants were gainfully employed. Higher parity was seen in those who developed breast cancer. Women with breast cancer also had higher levels of CRP and haptoglobin at baseline compared to those without. Participants with higher levels of CRP, haptoglobin, and WBC and lower albumin were older, less gainfully employed, and less likely to be nulliparous (Table 9).

Higher haptoglobin levels were associated with incident breast cancer (Table 10). This association slightly weakened in the age- adjusted and fully adjusted model, showing a borderline increased risk (HR: 1.09, 95% CI: 1.00-1.18) in the latter. No difference was observed between using age as a continuous or categorical variable. When the analysis was stratified based on age of 50 years as a proxy for menopause in the analysis with truncated follow-up and delayed entry (Table 11), a positive association was noted between CRP and breast cancer risk in premenopausal women (HR: 1.08, 95% CI: 1.08-1.30), whereas haptoglobin was associated with breast cancer risk only in postmenopausal women (HR: 1.09, 95% CI: 1.00-1.19). Similar patterns were observed with ordinary stratification analysis, although results were weaker for CRP. After adjustment for BMI in the subgroup of women with baseline BMI, the only association observed was between haptoglobin and incident breast cancer in postmenopausal women (HR: 1.24, 95% CI: 1.01-1.51 for high versus low levels of haptoglobin).

Table 8. Characteristics of study participants by breast cancer status

	Breast cancer (*N*=6,606)	**No breast cancer (*N*=148,573)**
Age (years) – Mean (SD)	50.33 (11.56)	46.26 (14.78)
Follow-up (years) – Mean (SD)	11.72 (5.48)	18.58 (4.43)
Parity – No (%)		
Nulliparous	1511 (22.87)	46781 (31.49)
1+	5095 (77.13)	101792 (68.51)
Socioeconomic status – No (%)		
White collar	2930 (44.35)	54935 (36.98)
Blue collar	3178 (48.11)	74558 (50.18)
Unemployed or unknown	498 (7.54)	19080 (12.84)
History of liver disease – No (%)	18 (0.27)	503 (0.34)
Body mass index (kg/m^2)[a]		
< 18.5	23 (2.05)	733 (3.08)
18.5-25	739 (65.86)	16002 (67.22)
25-30	262 (23.35)	5333 (22.40)
≥ 30	98 (8.73)	1737 (7.30)
CRP (mg/L) – No (%)		
< 10	5586 (84.56)	128354 (86.39)
≥ 10	1020 (15.44)	20219 (13.61)
Albumin (g/L) – No (%)		
< 40	880 (13.32)	17680 (11.90)
≥ 40	5726 (86.68)	130893 (88.10)
Haptoglobin (g/L)[b] – No (%)		
< 1.4	4118 (86.44)	84221 (87.60)
≥ 1.4	646 (13.56)	11918 (12.40)
WBC ($10^9/L$)[c] – No (%)		
< 10	2131 (94.08)	49762 (93.82)
≥ 10	134 (5.92)	3280 (6.18)

Measured in [a]24,297, [b]100,903 and [c]55,307 women.

Table 9. Distributions of inflammatory markers and demographical factors

	CRP (mg/L)		**Albumin (g/L)**		**Hp (g/L)**		**WBC(10^9/L)**	
	< 10	**≥ 10**	**< 40**	**≥ 40**	**< 1.4**	**≥ 1.4**	**< 10**	**≥ 10**
Age	46.06	48.78	51.36	45.77	46.02	51.56	49.07	48.09
	(14.51)	(15.49)	(16.19)	(14.35)	(14.18)	(14.50)	(16.89)	(16.35)
SES								
White collar	50567	7298	6229	51636	34129	3737	17468	988
	(37.75)	(34.36)	(33.56)	(37.80)	(38.63)	(29.74)	(33.66)	(28.94)
Blue collar	66873	10863	9158	68561	45674	6976	23445	1737
	(49.93)	(51.15)	(49.34)	(50.19)	(51.70)	(55.52)	(45.18)	(50.88)
Unemployed or	16500	3078	3173	16405	8536	1851	10980	689
unknown	(12.32)	(14.49)	(17.10)	(12.01)	(9.66)	(14.73)	(21.16)	(20.18)
Parity								
1+	92103	14784	13056	93831	61192	9224	34043	2302
	(68.76)	(69.61)	(70.34)	(68.68)	(69.27)	(73.42)	(65.60)	(67.43)

Table 10. Hazard ratios and 95% confidence intervals for breast cancer risk by levels of serum inflammatory markers in crude and individually adjusted models

	HR (95% CI)				
	Crude	**Age-adjusted**	**Age group-adjusted**[1]	**SES- adjusted**	**Parity-adjusted**
CRP (mg/L)					
< 10	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
≥ 10	1.03 (0.97-1.10)	0.98 (0.91-1.04)	0.99 (0.93-1.06)	1.04 (0.97-1.11)	1.03 (0.96-1.10)
WBC (10^9/L)					
< 10	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
≥ 10	0.99 (0.83-1.17)	1.06 (0.89-1.26)	1.03 (0.87-1.23)	1.0 (0.84-1.19)	0.98 (0.82-1.17)
Albumin (g/L)					
< 40	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
≥ 40	0.88 (0.82-0.94)	0.99 (0.92-1.06)	0.95 (0.89-1.02)	0.87 (0.81-0.93)	0.88 (0.82-0.95)
Haptoglobin (g/L)					
< 1.4	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
≥ 1.4	1.19 (1.10-1.30)	1.06 (0.99-1.16)	1.07 (1.00-1.17)	1.21 (1.11-1.31)	1.18 (1.08-1.28)

[1]Adjusted for age at baseline measurement as categories (<30, 30-40, 40-50, 50-60, 60-70, ≥70)

Table 11. Hazard ratios and 95% confidence intervals for breast cancer risk by levels of serum inflammatory markers in fully adjusted models

	N **breast cancer / *N* total**	**HR (95% CI)[a,b]**	**HR (95% CI)[a,c]**	***N*** **breast cancer / *N* total[b,d]**	**HR (95% CI)[b,d,e]**
All women					
CRP (mg/L)					
< 10	5586/133940	1.00 (Ref)	-	976/21972	1.00 (Ref)
≥ 10	1020/21239	0.99 (0.92-1.06)		146/2955	0.95 (0.80-1.14)
WBC (10^9/L)			-		
< 10	2131/51893	1.00 (Ref)	-	179/3881	1.00 (Ref)
≥ 10	134/3414	1.07 (0.90-1.28)	-	12/254	1.10 (0.61-1.99)
Albumin (g/L)					
< 40	880/18560	1.00 (Ref)	-	170/3370	1.00 (Ref)
≥ 40	5726/136619	0.97 (0.91-1.05)	-	952/21557	0.92 (0.79-1.09)
Haptoglobin (g/L)					
< 1.4	4118/88339	1.00 (Ref)	-	798/17587	1.00 (Ref)
≥ 1.4	646/12564	1.09 (1.00-1.18)	-	124/2233	1.20 (0.99-1.45)
Premenopause					
CRP (mg/L)					
< 10	2882/84511	1.00 (Ref)	1.00 (Ref)	559/14588	1.00 (Ref)
≥ 10	497/11663	1.18 (1.08-1.30)	1.02 (0.93-1.13)	79/1774	1.14 (0.90-1.44)
WBC (10^9/L)					
< 10	891/28729	1.00 (Ref)	1.00 (Ref)	95/2391	1.00 (Ref)
≥ 10	71/2073	1.04 (0.81-1.32)	1.07 (0.84-1.36)	6/164	0.84 (0.37-1.92)
Albumin (g/L)					
< 40	370/9241	1.00 (Ref)	1.00 (Ref)	91/2058	1.00 (Ref)
≥ 40	3009/86933	0.92 (0.83-1.02)	1.01 (0.90-1.12)	547/14304	0.87 (0.70-1.09)
Haptoglobin (g/L)					
< 1.4	2266/55976	1.00 (Ref)	1.00 (Ref)	482/11832	1.00 (Ref)
≥ 1.4	248/5956	0.94 (0.83-1.07)	0.93 (0.82-1.06)	56/1225	1.04 (0.79-1.38)
Postmenopause[d]					
CRP (mg/L)					
< 10	4730/109139	1.00 (Ref)	1.00 (Ref)	817/18767	1.00 (Ref)
≥ 10	893/18560	1.00 (0.93-1.07)	0.97 (0.88-1.07)	129/2633	0.99 (0.82-1.20)
WBC (10^9/L)					
< 10	1845/42027	1.00 (Ref)	1.00 (Ref)	155/3304	1.00 (Ref)
≥ 10	115/2810	1.06 (0.88-1.28)	1.02 (0.79-1.31)	9/231	0.95 (0.48-1.86)
Albumin (g/L)					
< 40	792/16325	1.00 (Ref)	1.00 (Ref)	145/2953	1.00 (Ref)
≥ 40	4831/111374	0.95 (0.88-1.03)	0.92 (0.84-1.02)	801/18447	0.93 (0.78-1.12)
Haptoglobin (g/L)					
< 1.4	3524/75302	1.00 (Ref)	1.00 (Ref)	666/15190	1.00 (Ref)
≥ 1.4	589/11617	1.09 (1.00-1.19)	1.19 (1.07-1.33)	114/2084	1.24 (1.01-1.51)

[a]Adjusted for age at baseline measurement (continuous), socioeconomic status, and parity

[b]Age of 50 years was used as a proxy for menopause. In the analysis of pre-menopausal women, individuals were followed to age 50 after which they were censored. In the assessment of post-menopausal risk, individuals with a baseline measurement taken before age 50 entered the study at age 50 by means of delayed entry. Note that this analysis allowed the same participants to be included in both groups, which resulted in a difference between the total numbers from pre-and post-menopausal analyses with the actual total numbers of women in the cohort.

[c]Stratification analysis by age of 50 years without truncated follow-up or delayed entry

[d]Subcohort analysis in women with baseline BMI

[c]Adjusted for age at baseline measurement (continuous), socioeconomic status, parity, and BMI

Figure 16 shows the proportions of breast cancer severity with respect to categories of prediagnostic CRP, albumin, haptoglobin and WBC, where no marked difference was observed between women with high and low levels of these markers. No association was found between these markers and the odds of being diagnosed with more severe breast cancer (Table 12).

High prediagnostic haptoglobin was linked to increased risk of dying from breast cancer (HR: 1.27, 95% CI: 1.02-1.59). A similar association was seen with CRP, but disappeared after adjustment for other covariates. For all-cause mortality, a stronger association with prediagnostic markers was found (Table 13). Women with higher levels of CRP, haptoglobin, and WBC are at greater risk of early death from any causes, with HR of 1.19 (95% CI: 1.04-1.36), 1.34 (1.15-1.55) and 1.57 (1.14-2.16) for high versus low levels of CRP, haptoglobin, and WBC, respectively. An inverse association for albumin was not apparent after adjustment for other covariates.

The association between prediagnostic serum inflammatory markers and death following breast cancer diagnosis was further demonstrated with cumulative incidence functions. A statistically significant higher cumulative risk of dying from breast cancer was observed in women with higher prediagnostic CRP and haptoglobin compared to those with lower levels (Figure 17). Similar but stronger trends were seen with all-cause death. Additionally, an inverse trend was observed between albumin and all-cause mortality over time. Although the hazard proportionality test showed no violation of the proportional hazard ($P>0.05$), cumulative incidence functions for WBC categories showed that hazard proportionality requirement was not met in longer follow-up. On the other hand, BCa survival by CRP categories was more evident in later follow-up, which may indicate limited power in the proportionality test with time-varying covariate.

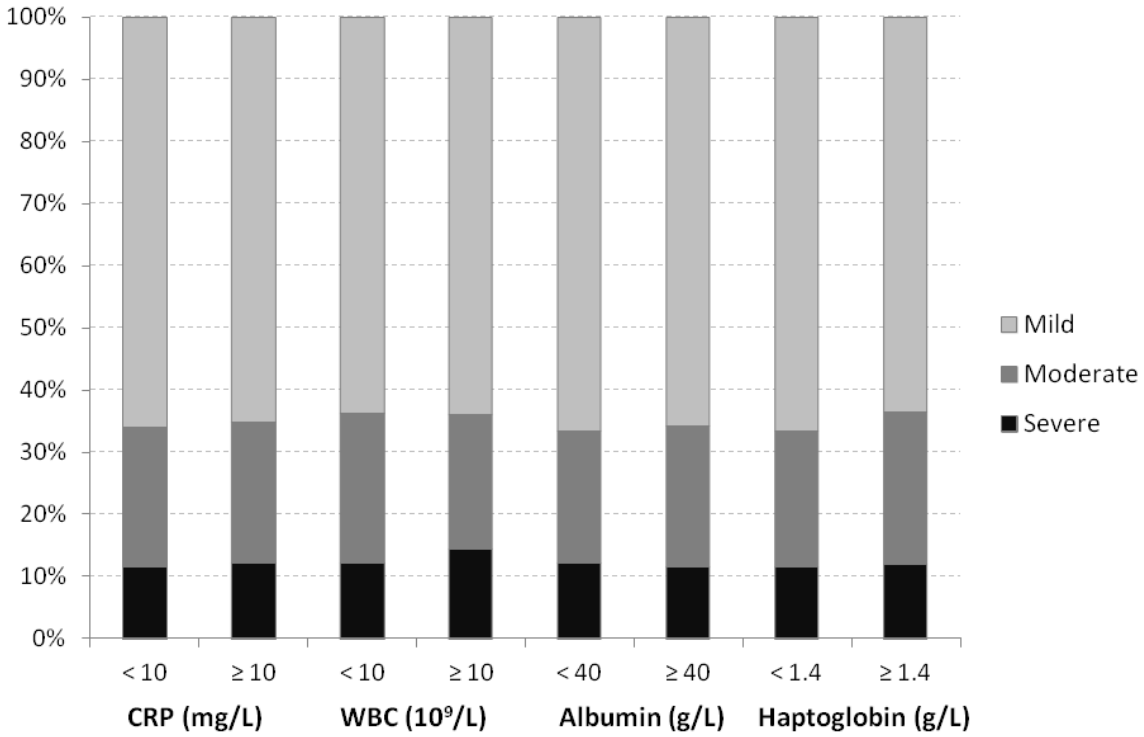


Figure 16. Proportion of breast cancer severity categories by levels of prediagnostic inflammatory markers

Table 12. Proportional odds ratios and 95% confidence intervals of more severe breast cancer by levels of prediagnostic serum inflammatory markers

	Proportional OR (95% CI)	
	Crude	**Adjusted[a]**
CRP (mg/L)		
< 10	1.00 (Ref)	1.00 (Ref)
≥ 10	1.04 (0.89-1.21)	1.04 (0.88-1.23)
WBC (10^9/L)		
< 10	1.00 (Ref)	1.00 (Ref)
≥ 10	1.03 (0.70-1.52)	1.08 (0.73-1.60)
Albumin (g/L)		
< 40	1.00 (Ref)	1.00 (Ref)
≥ 40	1.03 (0.87-1.21)	1.02 (0.86-1.22)
Haptoglobin (g/L)		
< 1.4	1.00 (Ref)	1.00 (Ref)
≥ 1.4	1.13 (0.93-1.37)	1.14 (0.94-1.39)

[a]Adjusted for age (continuous) and menopausal status at diagnosis, period of diagnosis, interval time between measurement and breast cancer diagnosis (continuous)

Table 13. Hazard ratios and 95% confidence intervals for deaths in breast cancer patients by levels of prediagnostic serum inflammatory markers

	N **death / *N*breast cancer**	**HR (95% CI)**	
		Crude	**Adjusted[a]**
Breast cancer-specific death			
CRP (mg/L)			
< 10	597 / 5584	1.00 (Ref)	1.00 (Ref)
≥ 10	139 / 1022	1.22 (1.01-1.46)	1.16 (0.95-1.41)
Albumin (g/L)			
< 40	113 / 878	1.00 (Ref)	1.00 (Ref)
≥ 40	623 / 5728	0.88 (0.72-1.08)	0.92 (0.75-1.13)
Haptoglobin (g/L)			
< 1.4	417 / 4107	1.00 (Ref)	1.00 (Ref)
≥ 1.4	95 / 655	1.31 (1.05-1.64)	1.27 (1.02-1.59)
WBC (10^9/L)			
< 10	240 / 2132	1.00 (Ref)	1.00 (Ref)
≥ 10	17 / 1332	1.23 (0.75-2.01)	1.23 (0.75-2.03)
All-cause death			
CRP (mg/L)			
< 10	1162 / 5584	1.00 (Ref)	1.00 (Ref)
≥ 10	312 / 1022	1.38 (1.21-1.56)	1.19 (1.04-1.36)
Albumin (g/L)			
< 40	256 / 878	1.00 (Ref)	1.00 (Ref)
≥ 40	1218 / 5728	0.78 (0.68-0.89)	0.95 (0.83-1.09)
Haptoglobin (g/L)			
< 1.4	914 / 4107	1.00 (Ref)	1.00 (Ref)
≥ 1.4	219 / 655	1.55 (1.34-1.80)	1.34 (1.15-1.55)
WBC (10^9/L)			
< 10	573 / 2132	1.00 (Ref)	1.00 (Ref)
≥ 10	42 / 132	1.25 (0.92-1.71)	1.57 (1.14-2.16)

[a]Adjusted for age (continuous) and menopausal status at diagnosis, TNM stage, ER status, period of diagnosis, interval time between measurement and breast cancer diagnosis (continuous)

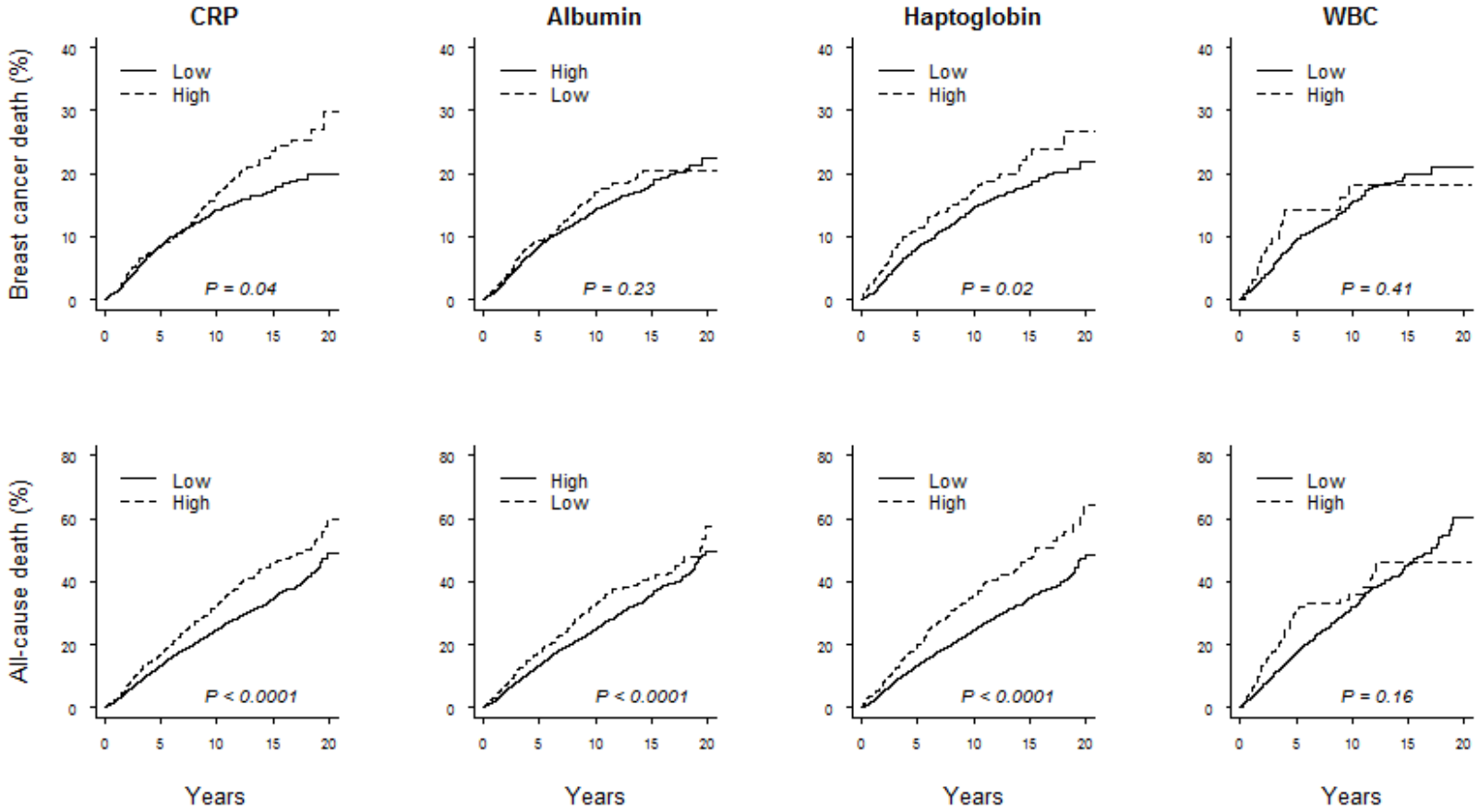


Figure 17. Cumulative mortality risk in breast cancer patients by levels of prediagnostic serum inflammatory markers. Note the different axes used in breast cancer-specific and all-cause death.

3.2. Atopy and Cancer

Characteristics of study participants by atopy status are shown in Table 14. The average age at baseline was 40 years, and more than half of the study population were female (59%). During a mean follow-up of 16 years, 689 persons were diagnosed with incident cancer. The most common three types of cancer were prostate, female breast, and colorectal cancers.

As shown in Figure 18, most individuals without atopy (specific IgE score of 0) had low or moderate levels of serum total IgE. The proportion of study participants with high total IgE increased with higher specific IgE scores, and nearly equalled total participants with specific IgE score of 5 and 6.

No association between atopy and risk of cancer was found (Table 15), but a statistically significant inverse trend was observed between highest serum specific IgE scores and risk of cancer in the overall study population and in women separately. Adjustment for serum total IgE showed stronger associations and an inverse association between atopy and cancer risk in the overall population. However, no marked difference in cancer risk by atopy status was noted in men and women separately. When analyses were stratified by serum total IgE levels, the inverse trend was only seen between serum specific IgE scores and cancer risk in the overall population and in women, but not men. No statistical significant interaction was found between serum total IgE categories and atopy or specific IgE scores ($P_{interaction}$ >0.05).

Using the models above with an additional adjustment for serum total IgE, the risk of the most common cancers was evaluated. Overall, no marked association was found between atopy and risk of specific cancers assessed (Table 16). When trends across specific IgE scores were observed, there was a lower risk of melanoma with higher specific IgE in both men and women combined (P_{trend} = 0.04). No clear association was observed for other cancer sites. In sex stratification, an inverse trend, albeit weak, was shown between specific IgE scores and melanoma risk in women (P_{trend} = 0.06).

Table 14. Characteristics of study participants by atopy status

	No atopy (N = 4,714)	**Atopy (N = 4,013)**
Age (years) – Mean (SD)	42.26 (13.74)	37.31 (12.66)
Sex, male – No (%)	1661 (35.24)	1945 (48.47)
Socioeconomic status – No (%)		
White collar	1912 (40.56)	1423 (35.46)
Blue collar	2074 (44.00)	1697 (42.49)
Unemployed/unknown	728 (15.44)	893 (22.25)
History of chronic respiratory disease – No (%)	67 (1.42)	92 (2.29)
Year of measurement – No (%)		
1992-1993	1192 (25.29)	986 (24.57)
1994-1996	2682 (56.89)	1997 (49.76)
1996	840 (17.82)	1030 (25.67)
Total IgE (kU/L) – No (%)		
< 25	1765 (37.44)	285 (7.10)
25-100	1937 (41.09)	1355 (33.77)
≥ 100	1012 (21.47)	2373 (59.13)
Mean follow-up (years) – Mean (SD)	15.86 (3.59)	16.05 (3.23)
Any cancer during follow-up – No (%)		
All cancer	443 (9.40)	246 (6.13)
Prostate	115 (2.44)	50 (1.25)
Breast (female)	55 (1.17)	42 (1.05)
Colorectal	43 (0.91)	21 (0.52)
Gynaecological	41 (0.87)	12 (0.30)
Haematological	31 (0.66)	22 (0.55)
Melanoma	27 (0.57)	10 (0.25)
Pulmonary	21 (0.45)	14 (0.35)
Bladder	14 (0.30)	11 (0.27)
CNS	11 (0.23)	9 (0.22)
Kidney	12 (0.25)	9 (0.22)

NMSC = nonmelanoma skin cancer; CNS = central nervous system

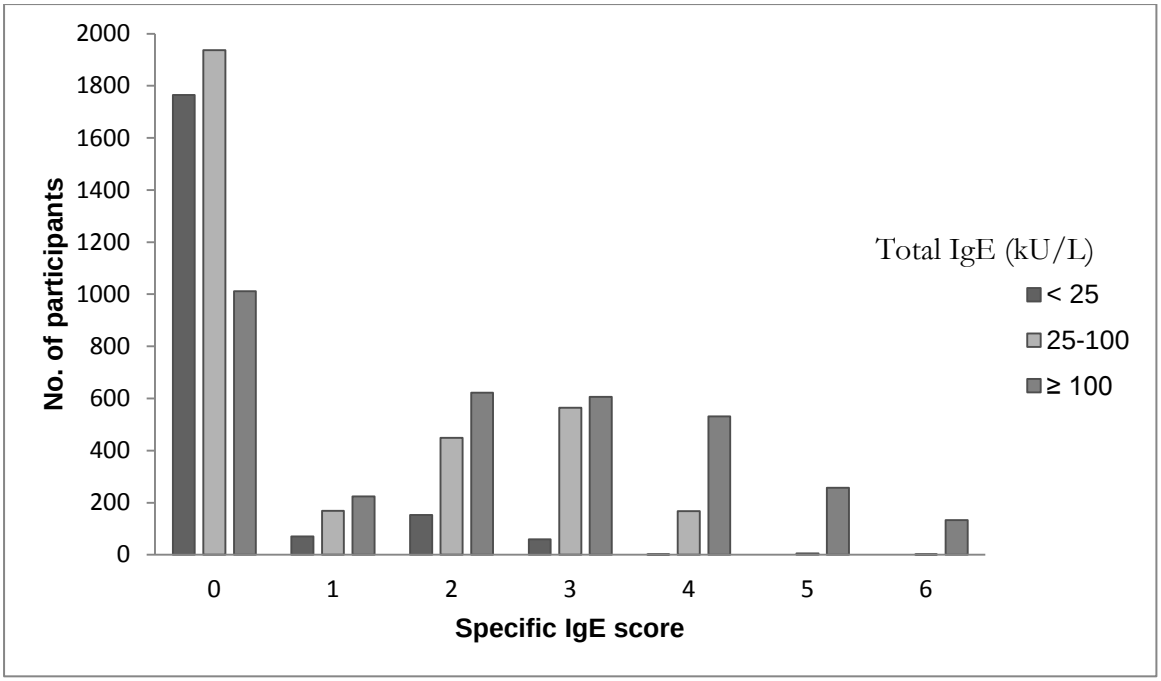


Figure 18. Distribution of participants by serum specific and total IgE categories

Table 15. Hazard ratios and 95% confidence intervals for incident cancer by atopy status and specific IgE scores

	N cancer/ *N* total	HR (95% CI)						
		Atopy		Specific IgE scores[a]				
		No atopy	Atopy	0	1-2	3-4	5-6	P_{trend}
Both men and women								
Multivariable model	689/8727	1.0 (Ref)	0.87 (0.74-1.02)	1.0 (Ref)	0.99 (0.82-1.20)	0.73 (0.58-0.91)	0.88 (0.53-1.46)	0.02
Additional adjustment for total IgE	689/8727	1.0 (Ref)	0.82 (0.76-0.99)	1.0 (Ref)	0.95 (0.77-1.16)	0.68 (0.53-0.87)	0.76 (0.45-1.29)	0.003
Stratification by total IgE (kU/L)								
< 25	191/2050	1.0 (Ref)	0.79 (0.48-1.31)	1.0 (Ref)	0.86 (0.51-1.47)	0.48 (0.12-1.96)	N/A	0.28
25-100	238/3292	1.0 (Ref)	0.84 (0.63-1.11)	1.0 (Ref)	0.91 (0.65-1.27)	0.75 (0.50-1.13)	N/A	0.14
> 100	260/3385	1.0 (Ref)	0.81 (0.63-1.04)	1.0 (Ref)	0.99 (0.74-1.33)	0.65 (0.47-0.89)	0.79 (0.46-1.35)	0.02
Men								
Multivariable model	277/3606	1.0 (Ref)	0.94 (0.74-1.20)	1.0 (Ref)	0.99 (0.74-1.34)	0.86 (0.63-1.18)	1.18 (0.59-2.35)	0.60
Additional adjustment for total IgE	277/3606	1.0 (Ref)	0.87 (0.67-1.13)	1.0 (Ref)	0.93 (0.68-1.27)	0.79 (0.57-1.11)	1.01 (0.49-2.06)	0.27
Stratification by total IgE (kU/L)								
< 25	53/649	1.0 (Ref)	0.70 (0.29-1.72)	1.0 (Ref)	0.59 (1.20-1.74)	1.08 (0.26-4.56)	N/A	0.61
25-100	97/1377	1.0 (Ref)	0.81 (0.53-1.25)	1.0 (Ref)	0.97 (0.58-1.64)	0.66 (0.36-1.19)	N/A	0.20
> 100	127/1580	1.0 (Ref)	0.94 (0.64-1.37)	1.0 (Ref)	0.99 (0.63-1.54)	0.86 (0.55-1.35)	1.14 (0.54-2.42)	0.79
Women								
Multivariable model	412/5121	1.0 (Ref)	0.82 (0.67-1.02)	1.0 (Ref)	0.98 (0.76-1.26)	0.63 (0.45-0.89)	0.71 (0.33-1.51)	0.01
Additional adjustment for total IgE	412/5121	1.0 (Ref)	0.80 (0.63-1.01)	1.0 (Ref)	0.96 (0.73-1.24)	0.60 (0.42-0.86)	0.63 (0.29-1.37)	0.009
Stratification by total IgE (kU/L)								
< 25	138/1401	1.0 (Ref)	0.82 (0.44-1.53)	1.0 (Ref)	0.98 (0.53-1.83)	N/A	N/A	0.30
25-100	141/1915	1.0 (Ref)	0.86 (0.59-1.25)	1.0 (Ref)	0.85 (0.54-1.34)	0.89 (0.51-1.55)	N/A	0.47
> 100	133/1805	1.0 (Ref)	0.73 (0.51-1.03)	1.0 (Ref)	0.98 (0.66-1.46)	0.51 (0.31-0.83)	0.62 (0.28-1.38)	0.01

[a]Highest specific IgE scores recorded at baseline

N/A = not applicable

All models used age as a timescale and were adjusted for sex (except for sex-specific analysis), socioeconomic status, period of measurement, and history of chronic pulmonary disease

Table 16. Hazard ratios and 95% confidence intervals for site-specific cancers by atopy status and specific IgE scores

	N cancer	HR (95% CI)						
		Atopy		Specific IgE scores[a]				
		No atopy	Atopy	0	1-2	3-4	5-6	P_{trend}
Both men and women								
All excluding NMSC[b]	664	1.0 (Ref)	0.82 (0.69-0.99)	1.0 (Ref)	0.96 (0.78-1.18)	0.67 (0.52-0.85)	0.81 (0.48-1.36)	0.005
Colorectal	64	1.0 (Ref)	0.70 (0.39-1.26)	1.0 (Ref)	0.75 (0.38-1.51)	0.59 (0.26-1.34)	1.22 (0.27-5.34)	0.31
Haematological	53	1.0 (Ref)	0.94 (0.51-1.75)	1.0 (Ref)	1.15 (0.58-2.29)	0.65 (0.27-1.58)	1.19 (0.27-5.38)	0.60
Melanoma	37	1.0 (Ref)	0.51 (0.23-1.15)	1.0 (Ref)	0.78 (0.33-1.86)	0.32 (0.09-1.10)	N/A	0.04
Pulmonary	35	1.0 (Ref)	0.87 (0.41-1.84)	1.0 (Ref)	1.24 (0.56-2.76)	0.40 (0.11-1.43)	0.94 (0.12-7.41)	0.22
Bladder	25	1.0 (Ref)	1.17 (0.49-2.81)	1.0 (Ref)	1.43 (0.55-3.73)	0.92 (0.28-3.04)	N/A	0.80
Kidney	21	1.0 (Ref)	1.08 (0.40-2.91)	1.0 (Ref)	1.49 (0.52-4.27)	0.26 (0.03-2.18)	3.28 (0.60-17.82)	0.98
CNS	20	1.0 (Ref)	1.30 (0.47-3.69)	1.0 (Ref)	1.81 (0.62-5.29)	0.86 (0.21-3.53)	N/A	0.71
Men								
All excluding NMSC[b]	267	1.0 (Ref)	0.89 (0.68-1.16)	1.0 (Ref)	0.95 (0.69-1.31)	0.80 (0.57-1.13)	1.12 (0.55-2.28)	0.37
Prostate	97	1.0 (Ref)	0.90 (0.58-1.40)	1.0 (Ref)	0.84 (0.49-1.45)	0.90 (0.51-1.56)	2.12 (0.72-6.27)	0.93
Colorectal	35	1.0 (Ref)	0.68 (0.32-1.44)	1.0 (Ref)	0.76 (0.31-1.86)	0.51 (0.18-1.44)	1.66 (0.34-7.99)	0.44
Haematological	23	1.0 (Ref)	1.12 (0.44-2.87)	1.0 (Ref)	1.10 (0.36-3.35)	1.11 (0.34-3.61)	1.33 (0.15-11.99)	0.79
Pulmonary	15	1.0 (Ref)	1.07 (0.35-3.26)	1.0 (Ref)	1.41 (0.42-4.71)	0.57 (0.11-3.02)	2.38 (0.24-23.99)	0.93
Melanoma	12	1.0 (Ref)	0.54 (0.15-2.00)	1.0 (Ref)	0.63 (0.13-3.12)	5.12 (0.10-2.68)	N/A	0.31
Women								
All excluding NMSC[b]	397	1.0 (Ref)	0.81 (0.63-1.03)	1.0 (Ref)	0.98 (0.75-1.28)	0.57 (0.39-0.83)	0.65 (0.30-1.42)	0.008
Breast	165	1.0 (Ref)	0.83 (0.57-1.20)	1.0 (Ref)	0.95 (0.62-1.44)	0.69 (0.40-1.19)	0.49 (0.12-2.03)	0.14
Gynaecological	53	1.0 (Ref)	0.55 (0.27-1.13)	1.0 (Ref)	0.54 (0.22-1.32)	0.65 (0.25-1.64)	N/A	0.11
Colorectal	29	1.0 (Ref)	0.76 (0.30-1.95)	1.0 (Ref)	0.75 (0.25-2.28)	0.87 (0.23-3.19)	N/A	0.56
Haematological	30	1.0 (Ref)	0.83 (0.37-1.87)	1.0 (Ref)	1.13 (0.48-2.70)	0.37 (0.08-1.63)	1.09 (0.14-8.74)	0.40
Melanoma	25	1.0 (Ref)	5.02 (0.18-1.38)	1.0 (Ref)	0.85 (0.30-2.39)	0.18 (0.02-1.45)	N/A	0.06

[a]Highest specific IgE scores recorded at baseline

N/A = not applicable; NMSC = nonmelanoma skin cancer; CNS = central nervous system

All models used age as a timescale and were adjusted for sex (except for sex-specific analysis), socioeconomic status, period of measurement, history of chronic pulmonary disease, and serum total IgE

[b]NMSC was excluded in this analysis due to underreporting in population cancer registries (283)

In our secondary analysis, we assessed risk of death following cancer diagnosis. As shown by the Kaplan-Meier curves in, the probability of survival was lower in men with IgE sensitisation compared to those without in the long term follow-up but there were no statistically significant difference (Log-rank P >0.05). Similarly, no differences were observed when categories of allergen-specific IgE scores were used (results not shown). We further evaluated this association by conducting Cox regression and found no clear associations between IgE sensitisation or specific IgE scores and death from all-causes or cancer, e.g. HR for cancer death was 1.04 (95% CI: 0.59-1.84) and 1.54 (0.91-2.62) for men and women with compared to without IgE sensitisation, respectively (results not shown in tables).

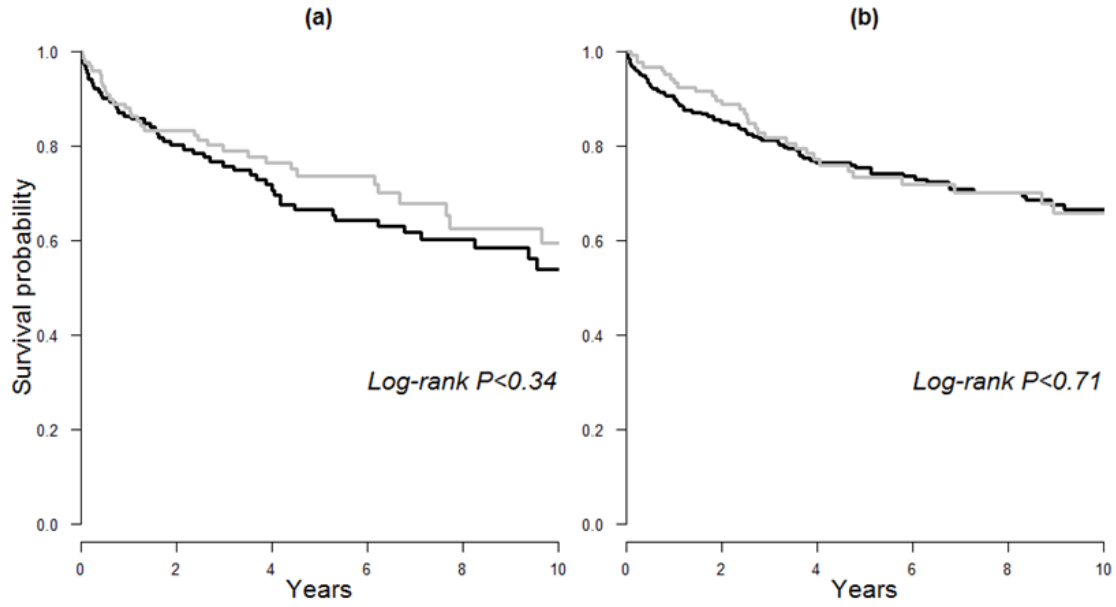


Figure 19. Kaplan-Meier curves of 10-year survival following cancer diagnosis in (a) men and (b) women based on prediagnostic IgE sensitisation. Thick lines indicate IgE sensitisation and the thin lines indicate a lack thereof.

3.3. Serum Lactate Dehydrogenase and Survival Following Cancer Diagnosis

Characteristics of study participants by LDH categories are shown in Table 17. Mean age at diagnosis was 62 years. At the end of follow up (mean: 8.2 years), 5,799 participants (73.5%) were deceased. Participants with high levels of baseline LDH (>ULN) were older, had higher co-morbidity burden, and lower 5-year overall survival rates. The three most frequent cancers were breast (female), prostate and colorectal cancer.

Overall survival differed by LDH measured within 3 years prior to diagnosis and within 3 months before date of diagnosis, i.e. LDH at time at diagnosis, with lower survival seen with higher LDH (Figure 20). Correspondingly, multivariable Cox proportional hazards regression showed an increased risk of dying from all causes with higher LDH z-score or categories, with a hazard ratio (HR) of 1.78 (95% CI: 1.64–1.94) comparing LDH levels above and below ULN. Similar findings were found when assessing cancer-specific death, e.g. the HR for overall cancer death was 1.85 (95% CI: 1.68–2.03) for high versus low LDH. Associations were slightly attenuated when the models were adjusted by cancer site Similar but more evident associations were found in the sub-analysis only including serum LDH at time at diagnosis (Table 18).

When specific cancer sites were assessed, higher risk of overall death was observed with high LDH in individuals diagnosed with breast, prostate, pulmonary, colorectal, gastroesophagealand haematological cancer and melanoma (Figure 21). The strongest association was seen for prostate cancer (HR: 2.19, 95% CI: 1.63–2.95). Similar but weaker trends were found when assessing cancer-specific death, with a positive association between LDH and risk of dying from prostate, pulmonary, colorectal, gastroesophageal, and haematological cancer. Additionally, a positive association was seen with gynaecological cancer death. In a subgroup analysis of 877 women with breast cancer and available information on tumour stage, adjustment for tumour stage did not alter the associations between LDH and death, with HR of all-cause and breast cancer death of 1.73 (95% CI: 1.12–2.67) and 1.54 (95%CI: 0.81–2.92), respectively, for high compared to low LDH levels.

Table 17. Characteristics of participants by categories of serum LDH measured within 3 years prior to cancer diagnosis

	LDH	
	≤ ULN (*N* = 7,216)	**> ULN (*N* = 679)**
Age at diagnosis (years) – Mean (SD)	62.29 (12.71)	65.62 (12.89)
Sex, male – No (%)	3682 (51.03)	381 (56.11)
Socioeconomic status – No (%)		
White collar	3136 (43.46)	237 (34.90)
Blue collar	2774 (38.44)	247 (36.38)
Unemployed or unknown	1306 (18.20)	195 (28.72)
Charlson comorbidity index – No (%)		
0	5871 (81.36)	525 (77.32)
1	871 (12.07)	82 (12.08)
2	252 (3.49)	37 (5.45)
3+	222 (3.08)	35 (5.15)
Period of diagnosis – No (%)		
Before 1989	1480 (20.51)	166 (24.45)
1989-1993	2215 (30.70)	235 (34.61)
1993-1997	2399 (33.25)	229 (33.72)
1997 onwards	1122 (15.56)	49 (7.22)
Cancer site – No (%)		
Breast	1081 (14.98)	37 (5.45)
Prostate	832 (11.53)	49 (7.22)
Pulmonary	598 (8.29)	70 (10.31)
Colorectal	784 (10.86)	82 (12.08)
Gastroesophageal	296 (4.10)	22 (3.24)
Hepatobiliary	130 (1.80)	36 (5.30)
Pancreas	222 (3.08)	35 (5.15)
Kidney	199 (2.76)	25 (3.68)
Bladder	335 (4.64)	14 (2.06)
Gynaecological	486 (6.74)	42 (6.19)
Head and neck	134 (1.86)	11 (1.62)
Melanoma	259 (3.59)	13 (1.91)
Central nervous system	302 (4.19)	14 (2.06)
Haematological	567 (7.86)	122 (17.97)
Survival time (months) - Median	67.81	13.72
5-year overall survival – No (%)	3760 (51.24)	169 (30.34)

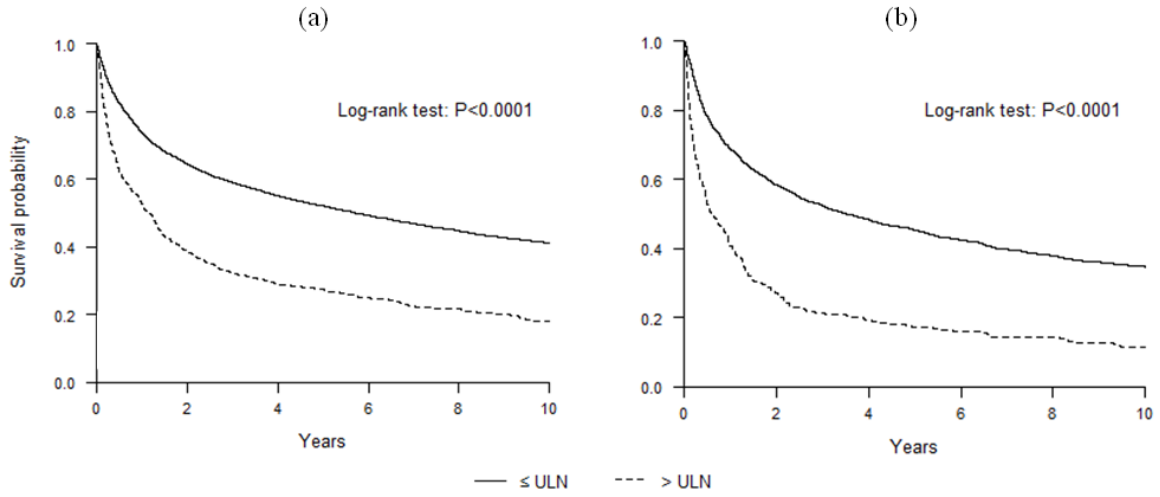


Figure 20. Kaplan-Meier curves for ten-year overall survival following cancer diagnosis by serum LDH levels measured (a) within 3 years prior to diagnosis, and (b) within 3 months prior to diagnosis

Table 18. Hazard ratios and 95% confidence intervals for death following cancer diagnosis by prediagnostic levels of LDH

		LDH	
	z-score	**≤ ULN**	**> ULN**
All-cause death			
N death/*N* total		5187 / 7216	612 / 679
Age-adjusted	1.18 (1.16-1.21)	1.0 (Ref)	1.78 (1.64-1.94)
Multivariable[a]	1.16 (1.14-1.19)	1.0 (Ref)	1.66 (1.53-1.81)
Adjusted by cancer site[a]	1.12 (1.10-1.15)	1.0 (Ref)	1.43 (1.31-1.56)
Sampling ≤ 3 months before diagnosis[b]	1.15 (1.12-1.18)	1.0 (Ref)	1.91 (1.65-2.20)
Cancer-specific death			
N death/*N* total		3760 / 7216	462 / 679
Age-adjusted	1.19 (1.17-1.22)	1.0 (Ref)	1.85 (1.68-2.03)
Multivariable[a]	1.17 (1.14-1.20)	1.0 (Ref)	1.72 (1.56-1.90)
Adjusted by cancer site[a]	1.12 (1.10-1.15)	1.0 (Ref)	1.46 (1.32-1.61)
Sampling ≤ 3 months before diagnosis[b]	1.17 (1.12-1.20)	1.0 (Ref)	2.06 (1.76-2.41)

[a]Adjusted for age at diagnosis (continuous), sex, socioeconomic status, Charlson comorbidity index, and period of diagnosis
[b]Subanalysis in 1,657 participants. Adjusted for age at diagnosis (continuous), sex, socioeconomic status, Charlson comorbidity index, period of diagnosis, and stratified by cancer site

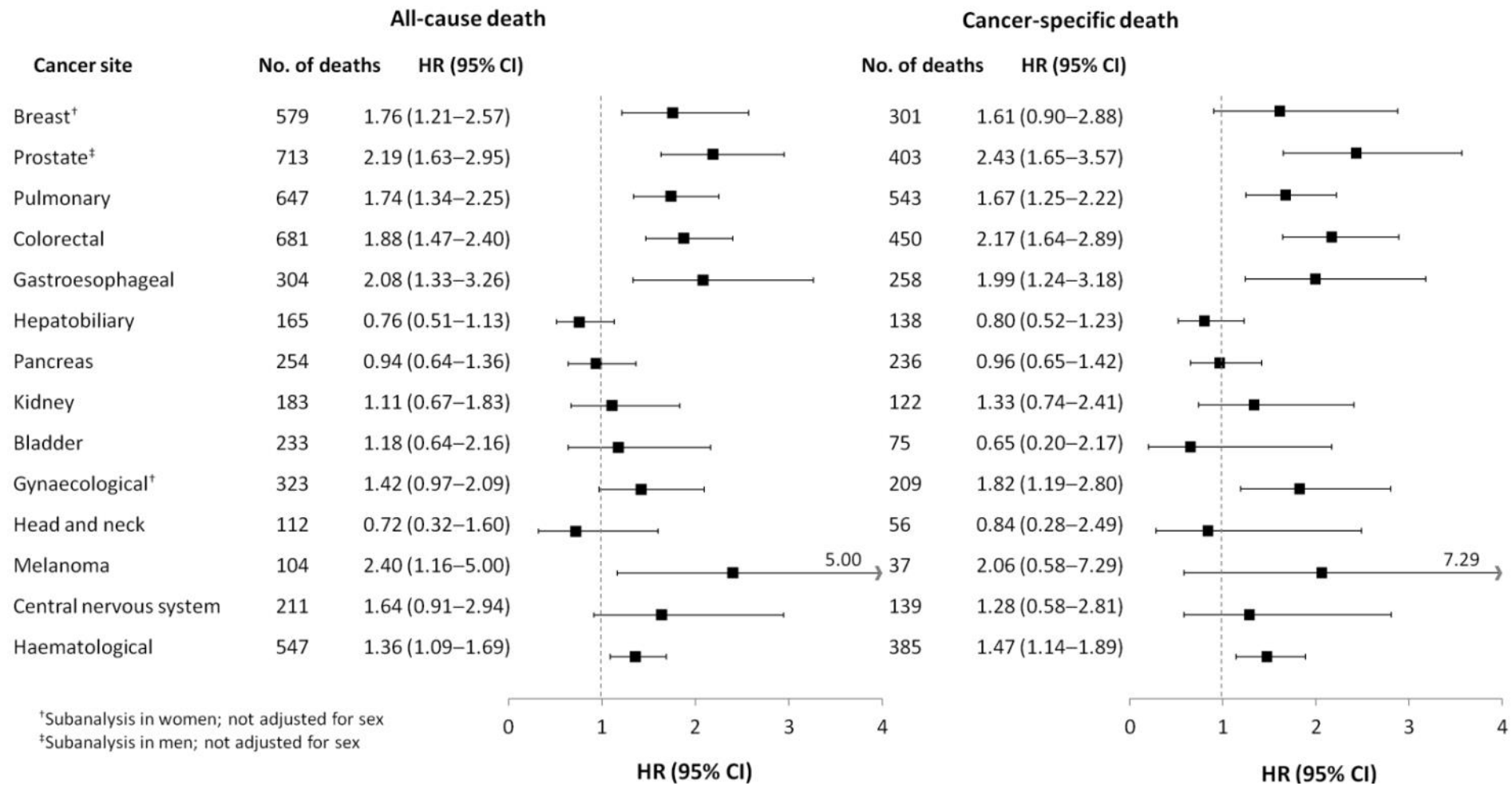


Figure 21. Hazard ratios and 95% confidence intervals for death following cancer diagnosis for high (> ULN) compared to low serum LDH (≤ ULN) as the reference, stratified by cancer site. All models were adjusted for age at diagnosis (continuous), sex, socioeconomic status, Charlson comorbidity index, and period of diagnosis.

The association between LDH and cancer was further visualised with cumulative incidence functions (Figure 22) and found higher cumulative risks of dying from overall, prostate, pulmonary, colorectal, gastroesophageal, kidney, gynaecological, and haematological cancer with high LDH levels. Interestingly, an inverse association was observed for head and neck cancer which approached statistical significance (Gray's test P = 0.05).

In the secondary analysis, LDH measured within six-month time intervals before cancer diagnosis was found to be increasing in interval times closer to diagnosis for overall and several types of cancer such as hepatobiliary and haematological cancer (Figure 23).

When risk of overall death in all participants was assessed for every interval time, a stronger association was observed with mean LDH measured closer to diagnosis, i.e. within 1 year prior to diagnosis. However, an increased risk of early death was found in those with high LDH measured 30 to 36 months prior to diagnosis (HR: 1.46, 95% CI: 1.15–1.86). For cancer-specific death, associations were also stronger when LDH was measured closer to diagnosis (Table 19). Similarly, in patients with stage I to II breast cancer, a positive association was observed between LDH measured 30 to 36 months before diagnosis and overall death (HR: 2.97, 95% CI: 1.38–6.39), and between LDH measured 6 to 12 months before diagnosis and breast cancer death (HR: 1.95, 95% CI: 1.24–16.00). Results in advanced stage of disease were hampered by a low number of events.

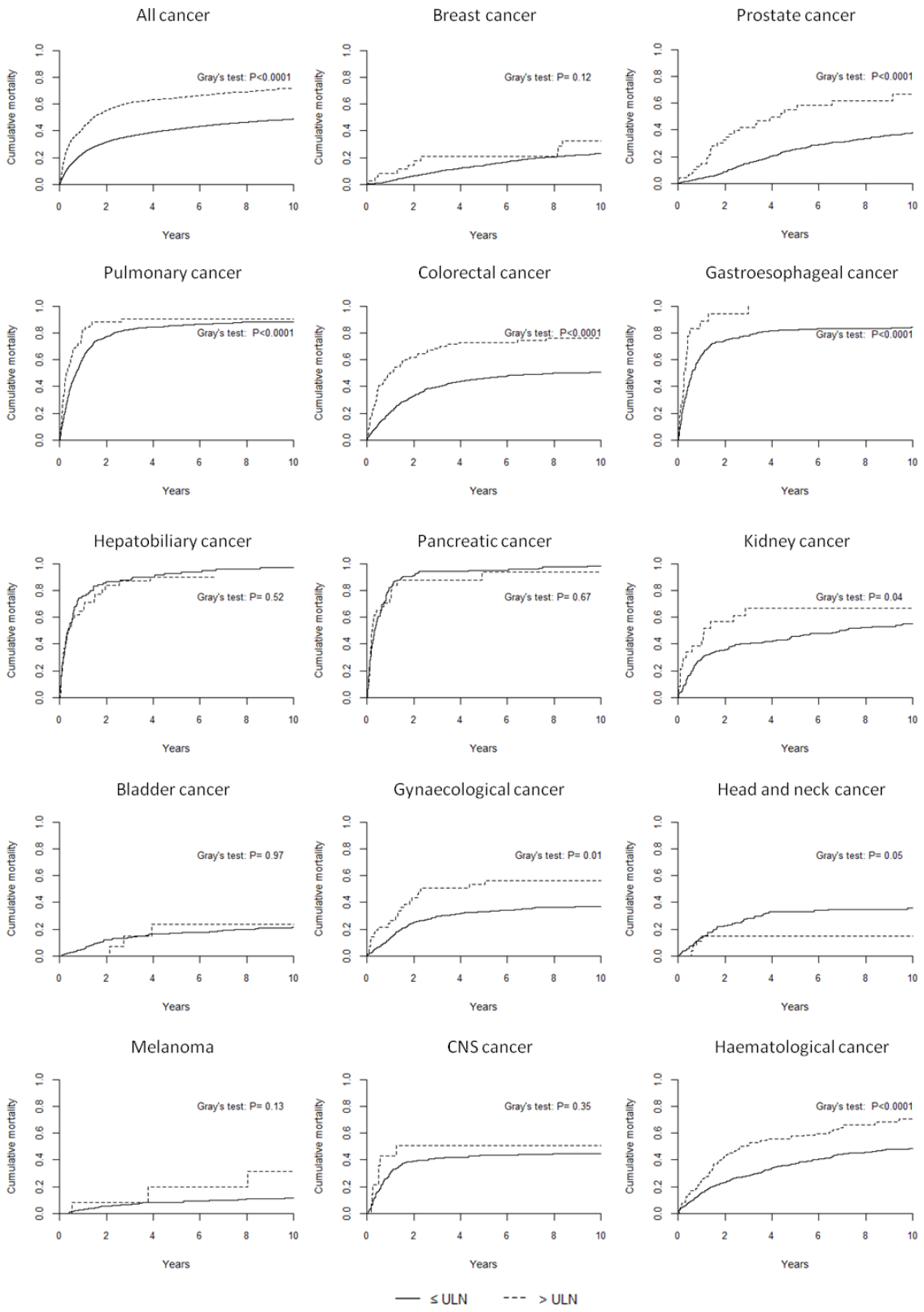


Figure 22. Ten-year cumulative incidence of cancer-specific deaths, stratified by clinical categories of serum LDH.

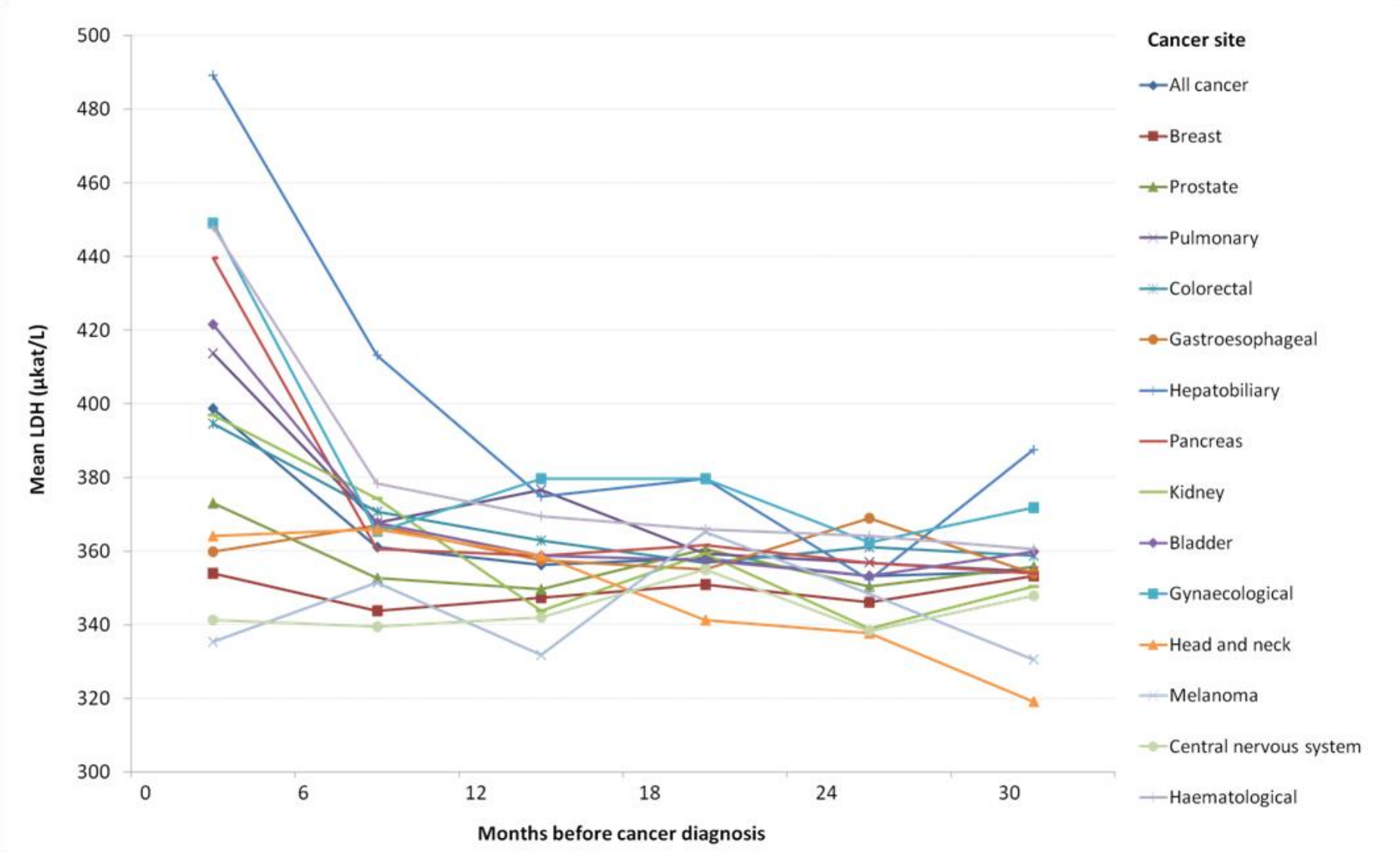


Figure 23. The average of serum LDH measured within 6-month interval times prior to cancer diagnosis, stratified by cancer site

Table 19. Hazard ratios and 95% confidence intervals for temporal associations between serum LDH and risk of death following cancer diagnosis.

Lag time[a], months	*N* death/*N* total	LDH[b]		
		z-score	≤ ULN	> ULN
All–cause death				
0–6	1952 / 2426	1.14 (1.11-1.17)	1.0 (Ref)	2.01 (1.79-2.25)
6–12	1124 / 1523	1.10 (1.03-1.18)	1.0 (Ref)	1.48 (1.21-1.83)
12–18	1188 / 1620	1.04 (0.96-1.12)	1.0 (Ref)	1.01 (0.79-1.31)
18–24	1176 / 1589	1.10 (1.02-1.18)	1.0 (Ref)	1.23 (0.96-1.57)
24–30	1287 / 1750	1.04 (0.96-1.13)	1.0 (Ref)	1.16 (0.90-1.51)
30–36	1300 / 1818	1.10 (1.01-1.19)	1.0 (Ref)	1.46 (1.15-1.86)
Cancer–specific death				
0–6	1498 / 2426	1.15 (1.12-1.18)	1.0 (Ref)	2.11 (1.86-2.40)
6–12	801 / 1523	1.11 (1.02-1.20)	1.0 (Ref)	1.54 (1.21-1.96)
12–18	839 / 1620	1.04 (0.95-1.15)	1.0 (Ref)	1.08 (0.80-1.47)
18–24	837 / 1589	1.07 (0.98-1.17)	1.0 (Ref)	1.07 (0.79-1.46)
24–30	913 / 1750	0.99 (0.90-1.10)	1.0 (Ref)	0.97 (0.68-1.38)
30–36	906 / 1818	1.04 (0.94-1.15)	1.0 (Ref)	1.10 (0.80-1.52)

[a]Interval time between baseline measurement of serum LDH and cancer diagnosis

[b]Average of serum LDH measurements taken within each interval time

All models were adjusted for age at diagnosis (continuous), sex, socioeconomic status, Charlson comorbidity index, period of diagnosis, and stratified by cancer sites

3.4. Associations of Serum Glucose and Lipids with Breast Cancer Death

At the end of follow up (mean: 13 years), a total of 861 (47.9%) study participants were deceased. Among these women, 425 died from breast cancer, 179 from cardiovascular disease, and 257 from other causes. The mean age of all participants was 58 at breast cancer diagnosis. Levels of glucose, triglycerides, and total cholesterol were highest in those dying from cardiovascular disease, whereas women who died from breast cancer had lower levels of the three markers compared to all women dying during follow-up period (Table 20).

When conventional Cox proportional hazards regression was performed, no strong association was observed between glucose, triglycerides, and total cholesterol and risk of dying from breast cancer (Table 21). On the other hand, positive associations were observed between triglycerides and cardiovascular death, as well as glucose and cardiovascular death. No association was observed for other causes of death. Proportions of deaths from each cause by quartiles of glucose, triglycerides, total cholesterol were displayed using the cumulative incidence functions. As shown in Figure 24, the proportion of women dying from cardiovascular disease markedly increased with higher quartiles of the markers, whilst deaths from breast cancer are less frequent with higher quartiles of the markers. This indicated cardiovascular death as a competing event.

The next analysis extended the proportional hazards model to include latent class variables and assess primary and non-primary outcomes. Bayesian model selection identified two latent classes in this study population. Retrospective analysis for class membership probability suggested that 81.5% women were more likely to be members of Class I, while the other 18.5% belonged to Class II. Baseline characteristics of study participants were further assessed in relation to the most probable latent class they were assigned to. Younger average age was observed in Class I compared to Class II, and a difference in socioeconomic status between classes was indicated (Table 22). With regards to clinical outcomes, no difference in proportions of women who died from breast cancer was found between the two classes. However, statistically significantly higher overall mortality rate from cardiovascular disease and other causes were seen in Class II.

Table 20. Characteristics of study participants overall and by causes of death

	All participants (n = 1,798)	Overall death (n = 861)	Breast cancer death (n =425)	Cardiovascular death (n=179)	Other death (n=257)
Age (years) – Mean (SD)	58.10 (11.82)	62.4 (13.22)	56.52 (12.47)	71.00 (10.25)	66.22 (11.38)
Follow-up time (years) – Mean (SD)	13.34 (6.90)	8.26 (5.91)	6.40 (5.04)	9.28 (6.47)	10.61 (5.96)
Interval between measurements and diagnosis (months) – Mean (SD)	18.26 (9.24)	18.10 (9.24)	18.36 (9.00)	17.64 (9.48)	17.88 (9.24)
Socioeconomic status					
White collar	648 (36.04	235 (27.29)	147 (34.59)	30 (16.76)	58 (22.57)
Blue collar	894 (49.72	405 (47.04)	222 (52.24)	61 (34.08)	122 (47.47)
Unemployed or unknown	256 (14.24)	221 (25.67)	56 (14.18)	88 (49.16)	77 (29.96)
Fasting status					
Fasting	1027 (57.12)	508 (59.00)	242 (56.94)	107 (59.78)	159 (61.87)
Not fasting	568 (31.59)	254 (29.50)	133 (31.30)	52 (29.05)	69 (26.85)
Unknown	203 (11.29)	99 (11.50)	50 (11.76)	20 (11.17)	29 (11.28)
Glucose (mmol/L) – Mean (SD)	5.10 (1.24)	5.25 (1.36)	5.04 (1.02)	5.46 (1.18)	5.44 (1.84)
Triglycerides (mmol/L) – Mean (SD)	1.27 (0.82)	1.40 (0.90)	1.30 (0.91)	1.62 (0.95)	1.42 (0.82)
Total cholesterol (mmol/L) – Mean (SD)	5.94 (1.17)	6.11 (1.24)	5.89 (0.53)	6.51 (1.21)	6.20 (1.19)

Table 21. Hazard ratios and confidence intervals for death from breast cancer, cardiovascular disease and other causes by levels of glucose, triglycerides, total cholesterol

		Breast cancer death			Cardiovascular death			Other death		
	N **total**	***N*** **event**	**HR[a]**	**95% CI**	***N*** **event**	**HR[a]**	**95% CI**	***N*** **event**	**HR[a]**	**95% CI**
Glucose, mmol/L[b]										
Continuous log			0.96	(0.58-1.59)		2.48	(1.24-4.96)		2.09	(1.16-3.76)
Quartiles										
<4.50	393	98	1	(Ref)	21	1	(Ref)	45	1	(Ref)
4.50-4.90	413	116	0.98	(0.75-1.29)	36	1.27	(0.74-2.19)	63	1.12	(0.76-1.64)
4.90-5.30	363	96	0.95	(0.72-1.27)	41	1.28	(0.75-2.19)	50	0.87	(0.58-1.30)
≥5.30	416	115	0.98	(0.74-1.29)	80	1.67	(1.02-2.73)	100	1.32	(0.92-1.89)
P_{trend}			0.83			0.03			0.20	
Triglycerides, mmol/L[c]										
Continuous log			1.21	(0.98-1.48)		1.58	(1.17-2.13)		1.32	(1.02-1.71)
Quartiles										
<0.70	297	81	1	(Ref)	12	1	(Ref)	24	1	(Ref)
0.70-1.00	491	102	0.77	(0.57-1.04)	34	0.91	(0.46-1.77)	56	0.96	(0.59-1.57)
1.00-1.60	555	132	0.97	(0.72-1.29)	52	1.10	(0.58-2.08)	95	1.28	(0.81-2.03)
≥1.60	455	110	1.05	(0.76-1.45)	80	1.53	(0.81-2.90)	83	1.22	(0.75-1.98)
P_{trend}			0.35			0.01			0.16	
Total cholesterol, mmol/L[d]										
Continuous log			0.72	(0.40-1.28)		2.04	(0.83-5.04)		0.67	(0.32-1.42)
Quartiles										
<5.20	443	119	1	(Ref)	16	1	(Ref)	38	1	(Ref)
5.20-5.80	403	94	0.87	(0.66-1.14)	37	1.52	(0.83-2.76)	60	1.18	(0.78-1.79)
5.80-6.60	470	102	0.79	(0.60-1.04)	40	1.26	(0.70-2.27)	75	1.06	(0.72-1.58)
≥6.60	482	110	0.85	(0.64-1.15)	85	1.74	(0.99-3.04)	85	0.92	(0.61-1.38)
P_{trend}			0.21			0.08			0.38	

[a]Adjusted for age at diagnosis (continuous), socioeconomic status, fasting status, glucose (continuous), triglycerides (continuous), and total cholesterol (continuous)
Not adjusted for [b]glucose, [c] triglycerides, [d]total cholesterol

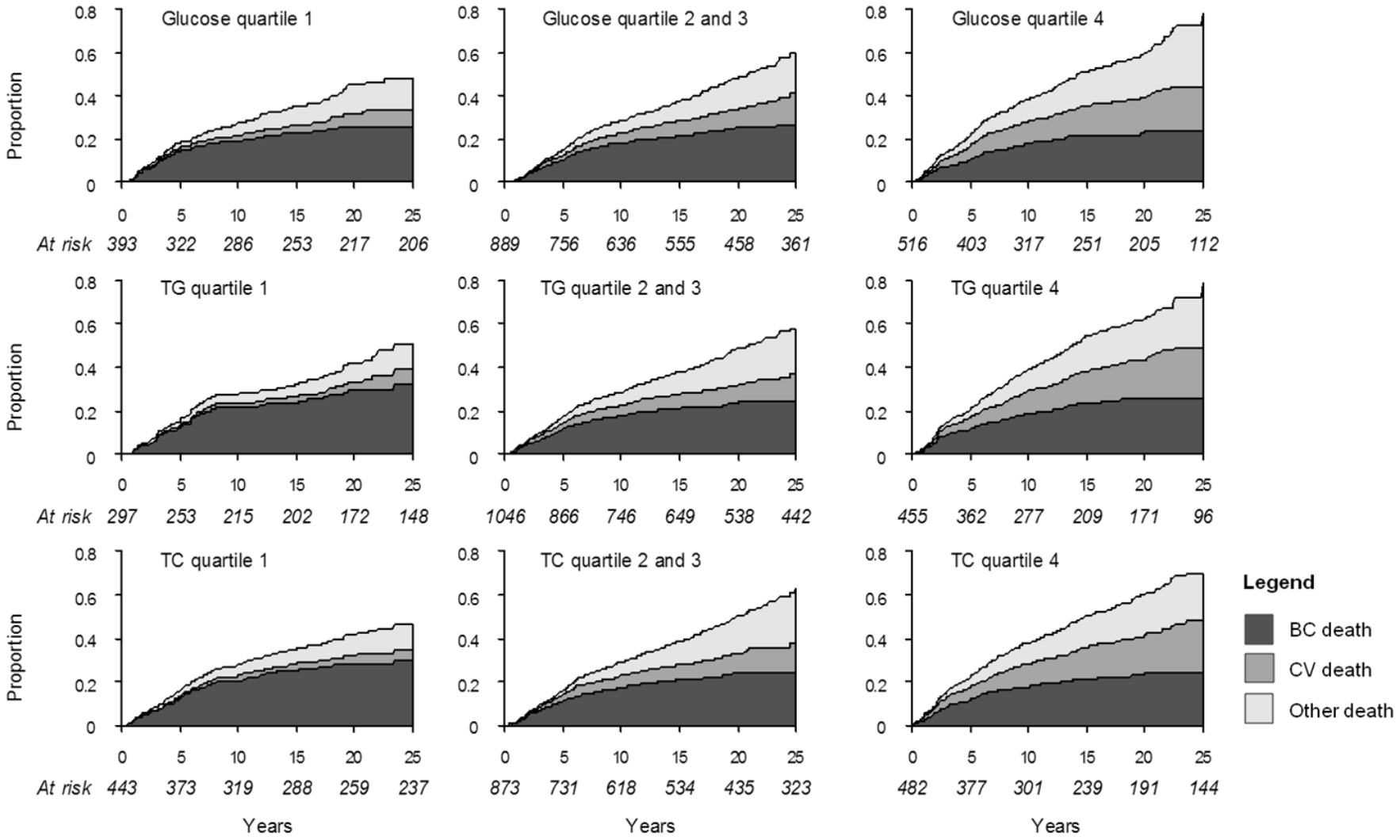


Figure 24. Stacked cumulative risk of death from breast cancer (BC), cardiovascular disease (CV), and other causes, stratified by quartiles of glucose, triglycerides (TG) and total cholesterol (TC).

Table 22. Characteristics of study participants and causes of death by predicted class membership

	Breast cancer patients		P-value
	Class I (*N* = 1,466)	**Class II (*N* = 332)**	
Age (years) – Mean (SD)	57.56 (10.91)	60.51 (14.99)	<0.0001
Socioeconomic status, No (%)			<0.0001
White collar	554 (37.79)	94 (28.31)	
Blue collar	739 (50.41)	155 (46.69)	
Unemployed or missing	173 (11.80)	83 (25.00)	
Fasting status, No (%)			0.55
Fasting	827 (56.41)	200 (60.24)	
Not fasting	477 (32.54)	91 (27.41)	
Missing	162 (11.05)	41 (12.35)	
Glucose (mmol/L) – Mean (SD)	5.12 (1.27)	5.00 (1.07)	0.08
Triglycerides (mmol/L) – Mean (SD)	1.26 (0.82)	1.31 (0.83)	0.32
Total cholesterol (mmol/L – Mean (SD)	5.93 (1.16)	6.00 (1.21)	0.34
Breast cancer death, No (%)	342 (23.33)	83 (25.00)	0.52
Cardiovascular death, No (%)	129 (8.80)	50 (15.06)	<0.0001
Other death, No (%)	60 (4.09)	197 (59.34)	<0.0001

The difference in survivals between latent classes was evaluated by displaying cumulative incidence functions for different causes of death by quartiles of glucose, triglycerides, and total cholesterol (Figure 25). Higher overall mortality was seen in Class II compared to Class I. In Class I, most patients died from breast cancer, whereas in Class II, most died from other causes apart from breast cancer and cardiovascular death. Increasing absolute numbers of deaths from breast cancer, cardiovascular, and other causes were seen with higher levels of all three markers in Class I, although there was no marked difference in relative mortality rates between each cause of death. On the other hand, marked differences in relative proportions of women dying from the three different causes were seen across levels of markers in Class II. For instance, breast cancer deaths were common amongst women in the lowest quartiles of glucose, triglycerides, and total cholesterol, but contributed little to total deaths in those with highest levels of the markers. More women died from cardiovascular disease with higher total cholesterol, and a similar association was seen between glucose and death from other causes.

Finally, the risk of different causes of death was assessed by obtaining class-specific hazard estimates. As seen in Table 19, log-transformed triglycerides corresponded to an increased risk of dying from breast cancer in Class I, with a hazard ratio of 1.87 (95% CI: 1.01-3.45). No statistically significant associations with breast cancer death were observed for other markers or among women in Class II. In agreement with class-specific cumulative incidence functions, women in Class II had a higher risk of cardiovascular death with higher total cholesterol and a higher risk of other death with higher glucose levels.

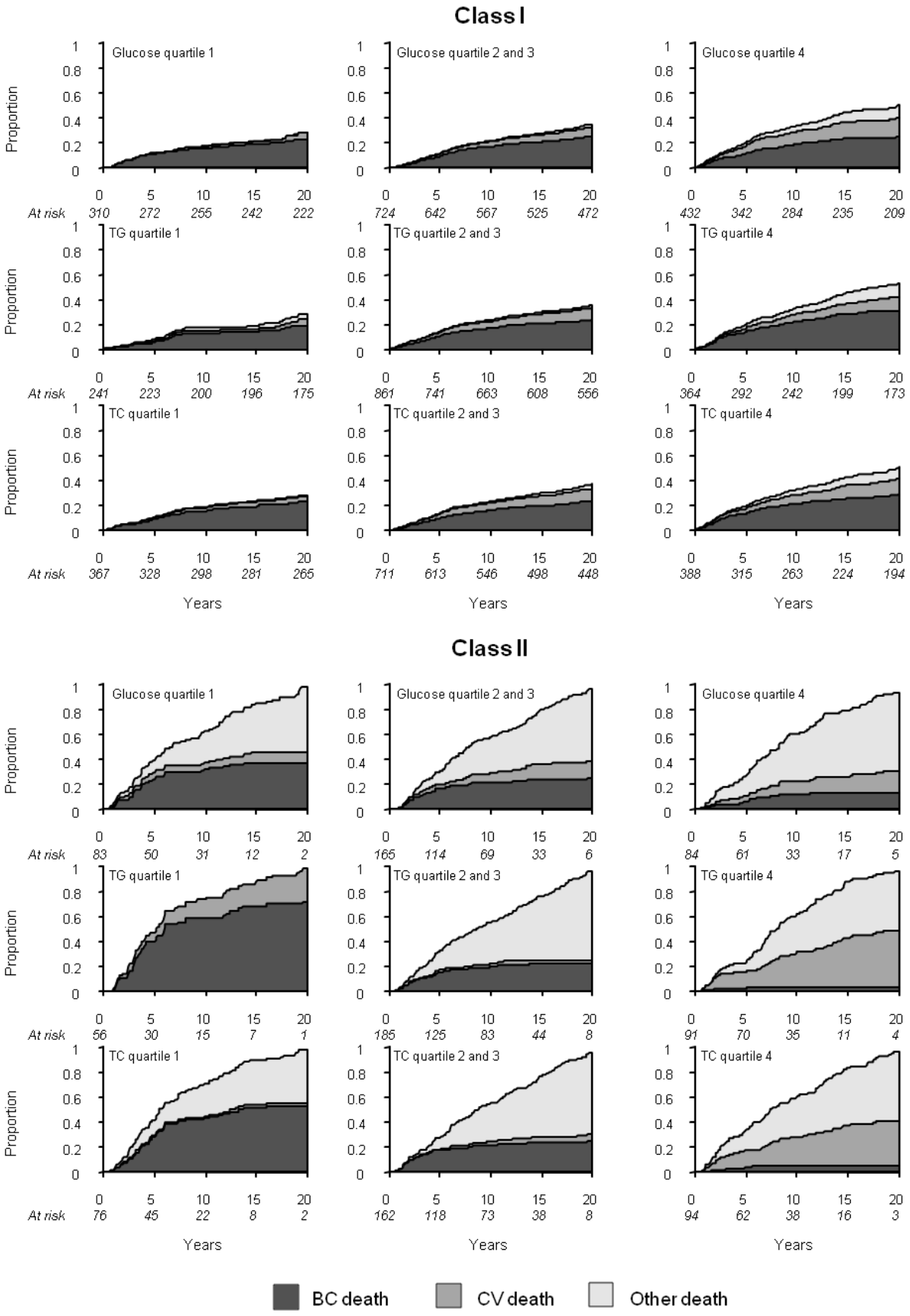


Figure 25. Stacked cumulative risk of death from breast cancer (BC), cardiovascular disease (CV), and other causes for each latent class, stratified by quartiles of glucose, triglycerides (TG) and total cholesterol (TC).

Table 23. Hazard ratios for death from breast cancer, cardiovascular disease and other causes by levels of glucose, triglycerides, total cholesterol for each latent class

	Class I		Class II	
	HR[a]	**(95% CI)**	**HR[a]**	**(95% CI)**
Breast cancer death				
Log glucose	1.09	(0.73-1.63)	0.84	(0.45-1.57)
Log triglycerides	1.87	(1.01-3.45)	0.91	(0.50-1.68)
Log total cholesterol	0.84	(0.49-1.45)	1.02	(0.53-1.99)
Cardiovascular death				
Log glucose	1.02	(0.55-1.91)	1.46	(0.97-2.20)
Log triglycerides	7.68	(2.45-24.02)	0.71	(0.40-1.25)
Log total cholesterol	0.86	(0.32-2.28)	2.07	(1.16-3.69)
Other death				
Log glucose	0.73	(0.50-1.05)	2.26	(1.50-3.40)
Log triglycerides	1.69	(0.95-3.01)	1.40	(0.74-2.64)
Log total cholesterol	1.20	(0.65-2.24)	0.45	(0.19-1.06)

[a]All covariates were included in a single model and adjusted for age at diagnosis (continuous), socioeconomic status and fasting status.

Chapter 4: Discussion

To further unravel the role of inflammation in breast cancer, this thesis utilised serum markers of inflammation as well as related metabolic and inflammatory factors in assessing the risk, severity and survival of breast cancer. This chapter summarises findings within this thesis and discusses them in relation to existing literature and future directions of research in similar fields. Similar to the Methods and Results, each research question is discussed separately.

4.1. Systemic inflammatory markers in relation to breast cancer risk, severity, and survival

A borderline positive association was found between baseline haptoglobin and incident breast cancer. Although no association was observed between inflammatory markers and breast cancer severity at diagnosis or ER positivity, haptoglobin was positively associated with breast cancer death. Breast cancer patients with higher levels of CRP or haptoglobin or lower albumin levels were also shown to be more likely to die early from any causes.

Molecular pathways linking inflammation and breast cancer have been increasingly studied. Pro-inflammatory cytokines released during inflammation may trigger the activation of signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappa B (NF-κB) signalling pathways, leading to activation of genes responsible for cell survival, proliferation, and angiogenesis (160, 161). Aberrant activation of STAT3 and NF-κB has been widely implicated in breast carcinogenesis (163, 292) and jointly contributes to an immunosuppressive tumour microenvironment (293). Additionally, suppressor of cytokine signalling 3 (SOCS3), an inhibitor of cytokine production, negatively regulates STAT3 expression and decreases proliferation in breast cancer cells, further linking inflammation and breast cancer (294). Apart from its effects on carcinogenesis, STAT3 upregulates expression of acute-phase reactants including CRP (295, 296) and haptoglobin (297), and a synergistic effect of NF-κB on this mechanism has been shown. These common regulatory pathways suggest that systemic markers of inflammation may be useful in studying the association between inflammation and breast carcinogenesis.

Analysis of CRP in relation to incident breast cancer showed a null association, which is in agreement with most previous studies (183, 184, 186, 188–190, 298, 299). So far, a positive association between serum CRP and breast cancer risk has only been documented in three

studies (187, 191, 300), where the largest number of breast cancer cases was 218. In addition to sample size, adjustments for potential confounding factors may explain the differences in estimates, especially BMI, since obesity has been suggested to underlie the association between chronic inflammation and breast cancer (301). Nevertheless, only one study showed substantial effect modification by overweight status (BMI $</\geq$ 25 kg/m^2) (299), whilst adjustment for BMI and other variables apart from age, such as hormonal factors, mostly had little impact on findings (184, 186, 187, 190, 300). Similarly, no marked difference was found in results when adjusting for BMI in the subset of women with baseline BMI, but the association between CRP and incident breast cancer in premenopausal women was no longer seen. This may imply that obesity-related inflammation in younger age plays a more important role in breast carcinogenesis compared to when it occurs later in life. However, there is not enough evidence suggesting that this could be translated to inflammation in general as no differences were seen for other markers upon BMI adjustment.

Despite the borderline association observed with incident breast cancer, risk estimates from haptoglobin were more robust than from CRP when adjusted for BMI, suggesting that the marker is less affected by obesity. So far, this is the first study exploring the association between haptoglobin and breast cancer in the population setting. Previously, haptoglobin has mostly been investigated in the context of breast cancer treatment, where a decreased serum expression in response to endocrine therapy was observed (302–304). In contrast, Dowling and colleagues compared the serum expression of haptoglobin in 33 breast cancer patients and 15 healthy females and found no statistically significant differences (305). The timing of measurements and the characteristics of participants, however, was not addressed. Given the paucity of evidence, these findings indicate a potential area of research with serum haptoglobin as a marker linking inflammation to incident and fatal breast cancer.

In this study, only prediagnostic haptoglobin showed a consistent association with breast cancer-specific death. Nevertheless, an increased risk of early death from all causes following breast cancer diagnosis was found in women with higher levels of CRP, haptoglobin and WBC, and a higher cumulative risk of all-cause mortality with lower albumin levels. This suggests better overall survival in breast cancer patients with normal levels of these markers prior to diagnosis. Allin and colleagues also studied prediagnostic CRP and all-cause mortality in 202 women diagnosed with breast cancer (189), and found no statistically significant association (HR: 1.1, 95% CI: 0.2-6.7 for CRP >3 mg/L compared to <1 mg/L, P_{trend} = 0.17). Besides the different

measurement methods, this discrepancy may be accounted for by the low number of cases in the previous study, rendering the risk difference between prediagnostic CRP levels unquantifiable. In addition, there is evidence that postdiagnostic serum CRP may be associated with worse cancer-specific and overall survival in breast cancer patients (306–309), which indicates a prognostic value of serum inflammatory markers in addition to their role in breast cancer aetiology as indicated in the present study. Future mechanistic investigations and clinical studies are thus necessary to explore their implication in underlying mechanisms of breast cancer and management of the disease.

To sum up, in addition to being weakly linked to incident breast cancer, higher prediagnostic serum haptoglobin levels were associated with a higher risk of death from the disease, which supports a role of inflammation in breast carcinogenesis. Additionally, women with higher prediagnostic CRP, haptoglobin or WBC levels are at higher risk of dying early from any causes following breast cancer diagnosis. These analyses imply that inflammation preceding breast cancer may impact survival after diagnosis. Such findings suggest the importance of inflammation as one of the mechanisms underlying breast cancer which may be further investigated for future intervention strategies in breast cancer patients.

4.2. Atopy and Cancer

In the present study, atopy was associated with a lower risk of incident cancer. The inverse trend between serum specific IgE was more pronounced in women and among those with high total IgE levels. Among the most common cancers, no association was observed except for an inverse trend between serum specific IgE scores and risk of melanoma in the overall population. A similar inverse but non-statistically significant trend was observed for breast cancer. No associations between prediagnostic allergen-specific IgE and survival following cancer diagnosis were observed.

A shift towards immunosuppressive immune responses is characteristic of cancer (13). Nevertheless, little is known about the role of humoral immune responses, particularly IgE, in carcinogenesis. IgE production and class-switch recombination (CSR) to IgE from other immunoglobulin types such as IgG are regulated by T_H2, and it has been suggested that a biased T_H2 response underlies high IgE levels in allergic individuals (310). Since only limited responses following allergen exposure are observed for IgE (311), it is possible that any impact on carcinogenesis is secondary to the biased T_H2 response rather than a result of high circulating IgE itself. In support of this, a temporal model of IgE and IgG has been proposed, in which the early-response IgE undergo sequential CSR to higher-affinity IgG3, then to IgG1, IgG2, and finally IgG4 (312–314), the latter of which displays low immunoactivatory functions. Furthermore, inflammatory T_H2-biased conditions such as IL-10, IL-4, VEGF and FoxP3+ Tregs that support class switching to IgG4 rather than IgE, and elevated IgG4 levels have been reported in different tumours including melanoma (315, 316). Specifically in melanoma, elevated serum IgG4 levels and IgG4+ B cells in patient circulation are associated with worse clinical outcomes, implying a bias towards an alternative rather than an IgE-biased response associated with melanoma cancer growth (317, 318). Taken together, these indications point toward a role of CSR dysregulation associated with T_H2-biased response in driving the link between allergy, circulating IgE, and IgG4-related diseases including some cancers (319).

So far, only few prospective studies reported the association between atopy according to serum specific IgE positivity and risk of cancer. In a recent study based on prospective cohorts in Denmark, Skaaby and colleagues analysed serum specific IgE of inhalant allergens in 14,849 individuals. A lack of association between atopy and risk of overall cancer was reported, with a HR of 1.00 (95% CI: 0.89-1.12) for atopy versus no atopy [19]. Similarly, a lack of a precise association when assessing atopy against inhalant allergens in relation to overall incident cancer

was found. However, taking into account serum total IgE showed an inverse association. Differences in follow-up periods and cohort composition may explain the discrepancy in the findings. The present study was based on a large cohort with a median follow-up of 18.6 years. In comparison, the study by Skaaby and colleagues comprised five cohorts spanning over different time periods, with a shorter overall median follow-up of 11.8 years (320). Although adjustments for other risk factors such as smoking, alcohol consumption and physical activity were performed in the previous study (320), they did not alter any findings and therefore they are unlikely to explain the discrepancy with the present study in which this information was unavailable.

For specific cancers, observational findings seem to vary by demographics and timing of specific IgE measurements. Two European nested case-control studies demonstrated an inverse association between atopy against inhalant allergens and risk of glioma in women but not men [20, 21], whereas no association was reported by a nested case-control study based on four U.S. cohorts [22]. Besides population attributes, a smaller number of cases in the latter study may explain such discrepancy. In case-control studies where specific IgE in cases was assessed after diagnosis, atopy against inhalant allergens was inversely associated with risk of lymphoid malignancies and positively with prostate cancer risk [23, 24]. However, no such associations were observed in studies where serum samples were prospectively collected before diagnosis [19, 23, 25].

In the present study population, no associations between atopy and risk of specific cancer sites were found which is comparable with the Danish study [19]. For breast cancer, a non-statistically significant inverse association between serum specific IgE scores and risk of breast cancer was observed in women. To date, evidence from observational studies on the role of atopy in breast remains unclear. In a meta-analysis, no associations between atopy or history of any allergy, asthma, or hay fever and breast cancer risk were suggested [8]. Interestingly, endocrine treatment agents for oestrogen-positive (ER+) breast cancers such as tamoxifen has been shown to reduce allergen-specific immunoglobulin levels including IgE in animal models of AD [30], which may suggest a potential interplay between immunologic and hormonal factors in breast cancer biology.

Additionally, higher scores of specific IgE were weakly associated with lower risk of melanoma when both men and women were assessed, although this association was weaker in sex-specific

analyses. To date, there have been no other studies investigating serum specific IgE in relation to melanoma risk, although results with other atopy assessments have been reported. Using skin prick test, two prospective cohorts found that atopy against inhalant allergens was not associated with subsequent melanoma incidence [9, 31]. On the other hand, findings based on history of atopic disorders have been conflicting. Hospitalisation with asthma has been linked to lower incidence of melanoma [29, 32], whereas both increased [33] and reduced risks [34] of melanoma were reported with atopic dermatitis. This again emphasises the need to refine quantitative assessments of atopy, which are able to both capture relevant immunologic response and clinical severity.

There are several caveats in assessing serum allergen-specific IgE as a marker of atopy. Allergen-specific IgE levels represent the probability of having clinical allergic disease, therefore, use of a single allergen and/or cutoff to define atopy may not fully be representative of one's allergic symptoms (321). In line with this notion, stronger associations were found with categories of specific IgE compared to the conventional single cutoff point of specific IgE levels, which indicates that specific IgE scores or categories may be more useful than a single cutoff point in assessing cancer risk. Additionally, there is an indication that the sum of specific IgE levels against common inhalant allergens correlates better with clinical symptoms such as wheezing (322) and hospitalisation with asthma (323), compared to individual levels of specific IgE. It was not possible to assess cumulative levels of specific IgE in this study. Therefore, future studies assessing allergy-related cancer susceptibility may benefit from refined criteria of atopy.

In summary, this study suggests that atopy is weakly associated with a lower risk of malignancy in cancer-free individuals. These findings add to the evidence that immune responses involved in allergy contribute to the susceptibility of being diagnosed with cancer, particularly melanoma. In particular, these results may support a role of T_H2-biased immune response in development of these cancers, indicated by a shift in the balance between circulating IgE and IgG subclasses including the low immunoactivatory IgG4, which urges further mechanistic investigations.

4.3. Serum Lactate Dehydrogenase and Survival Following Cancer Diagnosis

In the present study, higher prediagnostic LDH corresponded to lower overall and cancer-specific survival following cancer diagnosis. More specifically, a greater risk of dying from cancer was seen with increasing LDH in those diagnosed with prostate, pulmonary, colorectal, gastroesophageal, gynaecological or haematological cancer. Furthermore, associations between LDH and both all-cause and overall cancer deaths were stronger when LDH was measured closer to cancer diagnosis.

Several plausible mechanisms may underlie the link between LDH and cancer progression. Rapidly proliferating cancer cells requires extreme supplies of energy and chronic hypoxia secondary to tumour growth activates hypoxia-inducible factor 1 (HIF-1), a key regulator of glycolysis and angiogenesis (324). HIF-1 drives the metabolic switch to glycolysis by stimulating expression of glycolytic enzymes (221) and directly repressing mitochondrial function through activation of pyruvate dehydrogenase kinase 1 (PDK-1) (325, 326). The subsequent accumulation of glycolytic metabolites may promote further HIF-1 activation, resulting in a feed-forward stimulatory loop in cancer cells (220). HIF-1 also upregulates angiogenic factors including vascular endothelial growth factor-A (VEGF-A) (327), therefore linking glycolysis and LDH to angiogenesis and cancer progression (328, 329). However, continuous oxygen availability in glycolytic cancers, such as leukemia, suggests that other underlying factors may trigger the switch to aerobic glycolysis before hypoxia occurs (215). Additionally, the tumour-promoting role of A and B subunits of LDH has been suggested: increased LDH-A levels are crucial in c-MYC-mediated cell transformation (330, 331), whereas LDH-B is necessary in mammalian target of rapamycin (mTOR)-mediated tumourigenesis (332). These biological findings imply that LDH may be relevant to tumour growth and severity, and may also play a role in carcinogenesis. Further supporting this notion, recent evidence showed tumourigenesis secondary to a lack of oxidative phosphorylation in cells deficient in mitochondrial enzyme succinate dehydrogenase (333, 333, 334).

A prognostic value of serum LDH has been suggested in several types of cancer, particularly haematological malignancies. Serum LDH is a predictor of worse survival in diffuse large B-cell lymphoma (DLBCL) and is one of the five risk factors included in the International Prognostic Index (IPI) (229, 230). Similar associations with survival have been established in chronic myeloid and lymphocytic leukemias (234, 235) and small cell lung cancer (231, 233). In recent

clinical trials, elevated serum LDH has been shown as an independent predictor of overall survival in advanced or metastatic cancer of the breast (236), prostate (335, 336), colorectum (337), oesophagus (338), pancreas (339), ovary (340), nasopharynx (341, 342), gastric adenocarcinoma (343), hepatocellular carcinoma (344), renal cell carcinoma (345), and melanoma (346, 347). Summing up these studies, a recent meta-analysis demonstrated the inverse association between serum LDH and overall survival in solid tumours (237), although high publication bias was found.

Corroborating previous findings, the present study found higher risks of early death among cancer patients with high levels of baseline serum LDH at time of diagnosis and within 3 years before diagnosis. However, in the site-specific analysis with cancer-specific death as the outcome of interest, this association was only shown in those diagnosed with prostate, pulmonary, colorectal, gastroesophageal, gynaecological or haematological cancer. Considering the link of LDH with other chronic diseases which may contribute to death (238, 239), it is therefore important to consider cause-specific death to gain further insight into the prognostic relevance of LDH.

A borderline inverse trend was seen between LDH and head and neck cancer death, although the analysis was limited by the number of events. The unique distribution of LDH subunits (348) may explain different associations of serum expression of LDH with specific cancer types. Supporting this notion, an observation of average LDH levels measured by six-month intervals before diagnosis showed varying trend across different cancer sites, which may indicate a different extent of aerobic glycolysis with respect to cancer types. It is known that the majority of LDH subunits detected in the serum is LDH-B, although LDH-A exists in a lesser amount (226). The absence of *LDH-B* expression and its enzyme activities have been reported in cell lines of breast (349), prostate (350), gastric and pancreatic cancer (351), suggested to be driven by promoter hypermethylation. Since LDH-B kinetically favours the backward reaction of pyruvate-lactate conversion (348), this may suggest that LDH-A, which mostly catalyses the formation of lactate, is more relevant to cancer than LDH-B (226). However, recent evidence has shown that higher tissue expression of LDH-B correlates to overall survival in lung cancer and treatment response in breast cancer (352, 353), which highlights the role of LDH-B in cancer progression. Given the scarcity of data regarding the long-term impact of differential LDH expression on cancer survival, further investigations are needed to confirm the clinical usefulness of LDH with respect to its subunits or isoenzymes.

In addition to the positive association between prediagnostic LDH and death following cancer diagnosis, the importance of timing in LDH measurement was indicated in the present study. LDH measured within 12 months prior to the diagnosis of cancer was shown to be strongly associated with overall and cancer-specific death. This finding further indicates the relevance between LDH and tumour severity and may imply a predictive value of LDH for cancer diagnosis and severity. The positive association between LDH measured within 30 to 36 months before diagnosis and risk of overall as well as breast cancer death further signifies the importance of assessing cancer-specific death given the link between LDH and other fatal diseases such as cardiovascular disease (238).

Based on prospectively collected serum LDH, the present study demonstrated an inverse association between LDH and survival following diagnosis of several cancers, adding to the current evidence on the role of LDH in cancer progression. Future mechanistic studies are therefore necessary to establish whether serum LDH is a proxy of tumour growth and severity, which explains its association to cancer survival, or if it is also involved in early carcinogenesis.

4.4. Associations of Serum Glucose and Lipids with Breast Cancer Death

Survival analyses with Cox regression and a latent class proportional hazards model were performed to assess the association between prediagnostic markers of glucose and lipid metabolism and death from breast cancer in female breast cancer patients. The latter method accounted for cardiovascular death and other death as competing risks. With Cox proportional hazards model, a lack of association was observed between the three markers and breast cancer death. However, cardiovascular death was shown as a competing event. When latent class proportional hazards analysis were performed, two distinct latent classes were found within this cohort, reflecting different susceptibilities of dying from breast cancer based on their baseline characteristics. Class I, comprising the majority of the study population, was associated with an increased risk of breast cancer death following higher triglycerides levels. Overall survival was worse in Class II, among which higher total cholesterol levels were associated with an increased risk of cardiovascular death and higher glucose with risk of death from other causes. No association between the three markers and breast cancer death was seen in Class II.

Metabolisms of glucose and lipid have been implicated in many chronic diseases. In the context of cancer, an array of evidence has linked increased breast cancer incidence with aberrant levels of circulating glucose, triglycerides and total cholesterol at baseline (354–356). Abnormal levels of these markers are also associated with cardiovascular disease, which is the most common cause of death in general population (260, 261). This has also been demonstrated in the present study, as both glucose and triglycerides were associated with a higher risk of cardiovascular death, and the associations were stronger than those with breast cancer death. Several biological mechanisms are suggested to underlie this common link, such as chronic inflammation and insulin resistance, which may drive atherogenesis, cellular proliferation and angiogenesis (242, 262, 263). These shared metabolic pathways may thus result in a competing risks situation, where individuals with similar sets of risk factors are equally at risk of dying from both breast cancer and cardiovascular disease. In this case, a heterogeneous association between glucose and lipid markers and breast cancer death may be observed, which represents subpopulations or latent classes with different mortality risk profiles. However, this heterogeneity in survival data is not addressed by common analytical methods in cancer epidemiology.

Cox proportional hazards regression and latent classes proportional hazards model differ fundamentally in the assumptions made regarding risk correlations. In Cox, non-informative

censoring is assumed, which leads to the assumption of independence or no correlation between event times when multiple events are observed. However, in the real-world clinical observation, such assumptions are rarely assessable and sometimes inaccurate. The latent class proportional hazards model allows for the presence of heterogeneity underlying any observed risk associations (288) and predicts optimal parameters based on the most probable substructure of the study population. In the present study, this resulted in an optimal model with two latent classes. Overall survival was lower in Class II than Class I, which indicates the importance of taking into account risk associations when investigating biological markers in relation to cancer survival.

Triglyceride levels were associated with early death from breast cancer in Class I. This suggests an importance of lipid metabolism in disease progression in a relevant subset of breast cancer patients, which warrants further mechanistic investigation. No statistically significant association with breast cancer death was observed for glucose and total cholesterol, although among Class II they were associated with higher risks of dying from other causes and cardiovascular disease, respectively. Previous studies have reported a null association for triglycerides and total cholesterol in relation to all-cause mortality (357) and breast cancer-specific death (358), which is similar to the present findings using Cox regression and in Class II as assessed by latent classes proportional hazards model. Likewise, a lack of association with overall death has been reported for glucose(244, 245). Although Class I comprised the majority of all women studied, it is possible that the positive association between triglycerides and Class I was diluted in the overall cohort, resulting in a weaker association. Therefore, it is important to consider cohort heterogeneity in assessing this relationship.

Overall, there was a weak association between prediagnostic triglyceride levels and breast cancer death in the majority of women with breast cancer. On the other hand, glucose and total cholesterol were strongly associated with mortality from causes apart from breast cancer in the remaining patients, among which shorter overall survival was observed. The present study therefore demonstrated heterogeneity in the association between glucose, lipid markers, and breast cancer survival when cardiovascular death and other death were taken into account as competing outcomes. This implies an involvement of perturbed lipid metabolism in breast cancer progression and a complex interaction between baseline biological markers and co-morbidities in determining breast cancer survival which warrants mechanistic investigations. Therefore, such findings highlight the importance of considering cohort heterogeneity when evaluating biological markers in relation to cause-specific death.

Chapter 5: Conclusion

In this final chapter, the strengths and limitations of studies within this thesis are discussed, as well as their potential implications in future research in breast cancer aetiology.

Overall, the strength of studies conducted within AMORIS lies in the prospective evaluation of exposures and complete follow-up of study participants. All analyses were performed at the same laboratory with internationally accredited and calibrated methods (265). The population in AMORIS was selected by analysing fresh blood samples from health check-ups in non-hospitalised persons. However, any healthy worker effect would not influence the internal validity of the conducted studies. Information on race/ethnicity was not available, but the AMORIS cohort was similar to the general working population of Stockholm (359), which comprised about 80% Swedish-born individuals in 2000 (269).

The following section presents more detailed explanation on strengths and limitations which may affect interpretation of findings from each of the four studies.

5.1. Systemic inflammatory markers in relation to breast cancer risk, severity, and survival

This study assessed common inflammatory markers: serum C-reactive protein, white blood cells, albumin and haptoglobin in relation to breast cancer. Higher prediagnostic haptoglobin corresponded to higher breast cancer risk, and all markers were associated with survival following diagnosis.

To date, this is the largest prospective study assessing common serum inflammatory markers in relation to breast cancer. Nevertheless, there were some limitations regarding measurements of serum markers of inflammation. High-sensitivity CRP was not available at the time measurements were conducted. Thus, any CRP values below 10 mg/L were unquantifiable, which may have resulted in an underestimation of the association between serum CRP and breast cancer. Information on BMI was only available for a small proportion of the participants. However, a lack of effect of BMI on the association between serum inflammatory markers and breast cancer has been shown in previous studies (184, 186, 187, 190, 300).

Breast cancer is strongly affected by hormonal and reproductive factors, but in AMORIS there was no information on menopausal status or hormonal replacement therapy at baseline. However, these risk factors were taken into account by assessing parity and stratifying the analysis using age as a proxy for menopause. In the present study, higher parity was observed in women who had breast cancer, which opposes the well-accepted association between parity and breast cancer risk (96). Nevertheless, such discrepancy has been observed in several other Swedish cohorts (95, 360), and may be driven by differences in other socio-demographic factors beyond the scope of this thesis.

Finally, systemic inflammation has been linked to other causes of death such as cardiovascular disease (361), which may have driven the strong association with all-cause death and underestimated the effects of inflammatory markers on breast cancer-specific death.

5.2. Atopy and Cancer

This study demonstrated an inverse association between circulating IgE levels and incident cancer, which was driven largely by melanoma.

In addition to being able to document the role of atopy in cancer using both serum specific and total IgE, this is the first study investigating the impact of prediagnostic IgE levels on cancer survival. Therefore, the main strength of this study was the information on objective assessments of atopy prior to the diagnosis of cancer. Using age as a timescale addressed the strong influence of age on absolute levels of specific IgE and its relative proportion to total IgE (207, 284). A limitation of this study is the lack of information on clinical symptoms of allergy. Although information on specific types of allergens was available, it was not possible to link individual allergens with risk of cancer due to the lack of number of cases. Furthermore, continuous levels of serum specific IgE were not available and analytical methods differed with total IgE, rendering it impractical to quantify specific IgE activity using specific to total IgE ratio (207). Nevertheless, the present study was able to take into account total IgE in assessing cancer risk.

The population studied only included individuals who underwent IgE testing and therefore may not be representative of the general population. However, this is not expected to influence the internal validity of this study. Spurious correlations may be of concern when performing multiple comparisons as shown in our study. However, we planned our analyses based on prior evidence and our results are explicable by suggested biological pathways and findings from other studies. Therefore the observed association is unlikely to be spurious, although a discrepancy with the strength of the true association is possible due to the lack of cases. Allergy symptoms in participating individuals may have been confused with smoking-related respiratory disorders. To account for the lack of information on smoking, analyses were adjusted for history of hospitalisation with chronic obstructive pulmonary disease and asthma. Nevertheless, residual confounding may still have occurred. Lastly, analyses on specific cancer types and cause-specific deaths were limited by the number of cases and warrant confirmations by larger studies.

In summary, the associations observed between atopy and risk of cancer, particularly in women, may point towards a role of T-helper 2 (T_H2)-biased response in carcinogenesis. Considering the lack of mechanistic evidence, these findings signify a potential area for further studies.

5.3. Serum Lactate Dehydrogenase and Survival Following Cancer Diagnosis

Findings from this study demonstrated an inverse association of baseline serum LDH with overall survival from breast cancer, although a weaker association was observed with breast cancer-specific death.

Although a number of studies have indicated the association between LDH and overall survival (237), this is the first population-based study linking baseline LDH and cancer-specific survival. Moreover, prospectively collected LDH levels allowed an understanding into the relevance between levels of LDH and timing of cancer diagnosis. A limitation of this study is the lack of information on cancer treatment, and given the long period of recruitment (1986 to 1999), variation in management of cancer may affect timing of cancer diagnosis and its survival. Analyses were thus accounted for period of diagnosis in as a proxy for difference in screening and treatment over time.

Serum LDH increases due to other conditions such as myocardial infarction, inflammation and tissue injury (238–240), and therefore is not a specific marker of tumour development. Higher LDH at baseline may otherwise indicate inflammation or other disorders involves in pathways leading to cancer development. However, analyses were limited to three years prior to diagnosis to exclude reverse causation and were adjusted for CCI to take into account other diseases which may have predisposed one to worse survival. Additionally, there was no information on LDH subunits or isoenzymes and tumour characteristics such as stage, receptor status and histological grade. However, associations between LDH and all-cause or specific cancer death in breast cancer patients were not affected by tumour stage.

For lymphoma, the combination between serum LDH and tumour characteristics shows to be useful in predicting treatment response and prognosis (229). Future research should explore whether the role of LDH as a biomarker extends beyond breast cancer aetiology and encompasses any clinical relevance.

5.4. Associations of Serum Glucose and Lipids with Breast Cancer Death

Systemic inflammatory and metabolic factors have been linked to other causes of death such as cardiovascular disease (361), which may have driven the strong association with all-cause death and underestimated the effects of metabolic markers on breast cancer-specific death. This final study emphasised the importance of addressing cohort heterogeneity in relation to breast cancer survival in understanding the relationship between glucose and lipid markers and cause-specific death in presence of competing outcomes.

The strength of this study lies in the survival analysis method used to address competing risks. This is the first observational study utilising latent class proportional hazards model to address disease-specific survival in breast cancer, taking into account cardiovascular death and other death as competing events. As shown in this study, the advantage of incorporating latent class analysis and multiple events in addition to proportional hazards regression is that it allows identification of subpopulations within the cohort and final survival or hazard estimates of the primary event. In other words, this method may offer a suitable approach when dealing with survival functions or hazard rates estimation in presence of competing risks. A limitation of the present study was the lack of data representing older breast cancer patients, which may partly explain the low proportion of Class II. There was no information available on tumour characteristics, breast cancer susceptibility genes, and treatment or other metabolic and endocrine factors related to breast cancer such as obesity and use of hormonal replacement therapy. Although residual associations with unobserved covariates were captured by the model through identification of latent classes, underlying characteristics of these different subgroups of breast cancer patients may require further integration of other relevant markers or baseline information.

Although the latent class proportional hazards may offer a suitable approach to assess survival in presence of competing risks, understanding characteristics of different subgroups or latent classes of breast cancer patients may require further information on other relevant tumour markers or baseline patient characteristics.

5.5. Conclusions and Final Remarks

Breast cancer is very common, resulting in socioeconomic and psychological burdens which impact the overall population beyond those affected. Moreover, the ageing population worldwide adds to the burden of breast cancer because more women will be diagnosed with the disease. The high survivorship of breast cancer translates to approximately 8 out of 10 women predicted to survive their disease for at least ten years in developed countries. Consequently, there are also rising numbers of breast cancer survivors at risk of developing recurrence and other morbidities. This emerging view of breast cancer as a chronic disease stresses out the escalating disease burden posed by breast cancer due to an impending rise in proportions of survivors with disability. Thus, there is an increasing need to understand biological mechanisms involved in breast carcinogenesis to allow future development of more well-defined intervention.

By focusing on markers of inflammation, this thesis corroborates a role of inflammatory processes in the development and progression of breast cancer, which may have important implications in breast cancer research. More specifically, the four studies included in this thesis demonstrated that: 1) prediagnostic inflammation affected breast cancer incidence and affected survival, 2) there was an inverse association between atopy and cancer risk in women, although only weak associations were observed for breast cancer, 3) breast cancer patients with high serum LDH were at risk of early death, and 4) heterogeneous associations between triglyceride levels and breast cancer death were seen in presence of competing outcomes.

Taken together, findings from this thesis have provided more insight into the involvement of cellular and humoral immune responses in breast carcinogenesis, and indicated that inflammation taking place prior to cancer diagnosis may implicate the clinical course of the disease. This knowledge suggests that aetiological factors of breast cancer are of importance in the context of survival and warrant further investigations to explore any prognostic value. Furthermore, since breast cancer is a disease with long latency, associations between inflammatory markers observed in the longer-term prior to diagnosis, such as those observed with haptoglobin or specific IgE in this thesis, may imply causal contribution to carcinogenesis, whilst markers showing short-term association, such as LDH in this thesis, may be related to early stages of pre-clinical cancer due to "reverse causality". They may be of potential interest for early cancer diagnosis. Therefore, further studies investigating temporality of associations between inflammation and breast cancer may further identify their clinical or public health importance. Finally, from methodological point of view, this thesis combined conventional approaches such as Cox regression, and more

recent approaches such as the latent class proportional hazards. Careful considerations should be made in selecting the most appropriate methods, preferably at the stage of study design. Conventional survival analysis techniques such as Kaplan-Meier survival function and Cox regression have straight-forward interpretation and widely used due to their practicality, thereby allowing easy comparability between studies. However, certain assumptions need to be met for the analyses, which often contradict real-life situation. More complex analytical methods may liberate one from such assumptions, at the price of more challenging interpretation and comparability. For instance, the latent class proportional hazards employed in this thesis allowed insight into risk correlations between breast cancer and other outcomes, a real-life situation which is not easily unpicked with classic survival analyses. The observed heterogeneity implies promising benefit in improving risk stratification of breast cancer patients. However, further understanding of characteristics of subpopulations identified by the model, as well as robustness ascertainment of models against more complex clinical characteristics are essential to take forward these findings to any meaningful empirical application.

In summary, findings from this study add to the knowledge of and inform scientific community regarding the implication of immune-related biological mechanisms in breast carcinogenesis, and lead to new avenues of research to explore any potential clinical importance of inflammatory and metabolic markers in breast cancer development and progression.

Chapter 6: References

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Appendices

Appendix A. Main Publications

Appendix A1. Metabolic serum biomarkers for the prediction of cancer: a follow-up of the studies conducted in the Swedish AMORIS study. Published in ecancermedicalscience, 2015.

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Metabolic serum biomarkers for the prediction of cancer: a follow-up of the studies conducted in the Swedish AMORIS study

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Abstract

The Swedish Apolipoprotein MOrtality RISk study (AMORIS) contains information on more than 500 biomarkers collected from 397,443 men and 414,630 women from the greater Stockholm area during the period 1985–1996. Using a ten-digit personal identification code, this database has been linked to Swedish national registries, which provide data on socioeconomic status, vital status, cancer diagnosis, comorbidity, and emigration. Within AMORIS, 18 studies assessing risk of overall and site-specific cancers have been published, utilising a range of serum markers representing glucose and lipid metabolism, immune system, iron metabolism, liver metabolism, and bone metabolism. This review briefly summarises these findings in relation to more recently published studies and provides an overview of where we are today and the challenges of observational studies when studying cancer risk prediction.

Overall, more recent observational studies supported previous findings obtained in AMORIS, although no new results have been reported for serum fructosamine and inorganic phosphate with respect to cancer risk. A drawback of using serum markers in predicting cancer risk is the potential fluctuations following other pathological conditions, resulting in non-specificity and imprecision of associations observed. Utilisation of multiple combination markers may provide more specificity, as well as give us repeated instead of single measurements. Associations with other diseases may also necessitate further analytical strategies addressing effects of serum markers on competing events in addition to cancer. Finally, delineating the role of serum metabolic markers may generate valuable information to complement emerging clinical studies on preventive effects of drugs and supplements targeting metabolic disorders against cancer.

Keywords: *cancer, serum lipids, serum glucose, C-reactive protein, leukocytes, IgE, calcium, iron, gamma-glutamyl transferase*

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Introduction

The Swedish AMORIS database is by far one of the largest prospective cohort studies with detailed information on serum biomarkers. Between 1985 and 1996, the Central Automation Laboratory collected and analysed blood samples of 397,443 men and 414,630 women, mainly from the greater Stockholm area [1–4]. All individuals were either healthy individuals referred for clinical laboratory testing as part of a general health checkup or outpatients. This database with information on >500 biomarkers has been linked to several Swedish national registries such as the National Cancer Register, the Patient Register, the Cause of Death Register, the consecutive Swedish Censuses during 1970–1990, and the National Register of Emigration. By using the Swedish ten-digit personal identity number one can get information on socioeconomic status, vital status, cancer diagnosis, comorbidity, and emigration.

With respect to cancer outcomes, 18 studies to date investigated the association with serum biomarkers of lipid and glucose metabolism, the immune system, liver metabolism, iron metabolism, and bone metabolism in AMORIS [5–22]. Following a brief overview of the results found for all biomarkers studied in AMORIS, the current review aims to summarise subsequently published epidemiological evidence on these serum biomarkers in relation to risk of cancer development.

Literature review

For each following subsection we used related medical subject headings (MeSH) terms for the biomarkers studied in AMORIS as well as 'neoplasm'. Both PubMed and Embase were searched only using the date of AMORIS publications as a limitation to ensure that we found all epidemiological evidence published subsequently to our findings in this Swedish prospective cohort. Studies relevant to previous work in AMORIS were selected and included in this review.

Lipid metabolism

Selected biomarkers

A wide variety of serum biomarkers allow the investigation into the association between lipid metabolism and cancer. **Triglycerides** constitute the majority of the lipids in the body, whereas **cholesterol** is a precursor for plasma membranes, bile salts, steroid hormones, and other specialised molecules. Cholesterol requires lipoproteins to be transported in the blood stream. **Low density lipoproteins** (LDL) are the main cholesterol carriers and they deliver cholesterol to cells throughout the body [23]. In contrast, **high-density lipoproteins** (HDL) remove excess cholesterol from blood and tissue. **Apolipoproteins A-I and B** (ApoA-I and ApoB) are structural proteins of these lipoprotein particles assisting in their transport [24].

Dyslipidaemia, or abnormal lipid metabolism, is thought to be involved in cancer development through a pathway linked to fatty acid synthesis [25–29]. High serum levels of lipid components such as triglycerides, total cholesterol, LDL, and ApoB have also been implicated in development of certain types of cancers such as breast and prostate by stimulating the Akt and AMPK pathways, which are associated with DNA damage and cell proliferation [30–32]. Additionally, hypercholesterolaemia has been shown to up-regulate the activity of transcriptional factors such as Sterol Regulatory Element-Binding Proteins (SREBP) and low-density lipoprotein receptor (LDLr), which promote carcinogenesis [33, 34]. All these evidence suggests a potential role of serum lipids in the prediction of cancer.

Findings in AMORIS

We have studied the interplay between glucose, triglycerides, total cholesterol and the associated risk of prostate, kidney, and gastrointestinal cancers [10, 11, 14, 15]. Our findings supported the hypothesis that components from the lipid metabolism influence risk of developing cancer, although a greater risk of prostate cancer with increasing triglycerides was only seen in men with higher glucose levels [11].

Low levels of HDL and ApoA-I were also found to be associated with increased prostate cancer risk [14]. Additionally, we studied the link between serum lipids and risk of breast, endometrial, and ovarian cancer [7, 8], and found a positive association between serum triglycerides and risk of endometrial cancer, whereas only a weak inverse relation was observed for breast cancer.

New epidemiological findings in the literature

Since the last AMORIS publication, several epidemiological studies have also focused on serum lipid markers and risk of prostate cancer (Table 1). A statistically significant positive association was observed with total cholesterol [35–38], whereas an inverse association was found for triglycerides [39]. When focusing specifically on aggressive prostate cancer, the Cancer Prevention Study II Nutrition Cohort [40] reported that neither total cholesterol, LDL- or HDL-cholesterol were associated with it. Also for gastrointestinal cancers, many more studies have been published. Total cholesterol and triglycerides have been positively associated with risk of colorectal cancer [41, 42], whereas HDL has been found to either have no effect or reduce this risk [43]. Most studies failed to demonstrate any effect of circulating lipids on risk of rectal cancer alone [43–45].

In addition, an increased risk for breast, bladder, and pancreatic cancer has been observed among those with high circulating levels of total cholesterol, triglycerides, LDL, and low circulating levels of HDL [35, 46–49] compared to those with normal levels. In contrast, no statistically significant association was found between lipid components and risk of ovarian cancer in the Metabolic syndrome and Cancer project (Me-Can) [50]. Similarly, null-findings were observed in a prospective cohort study based on a Korean population focusing on cervical, kidney, gall bladder, pancreatic, lung, and oesophageal cancers. However, in the same study when authors analysed serum lipid levels and the associated risk of stomach and liver cancer, they found an inverse association [35]. With respect to the inverse association between ApoA-I and cancer, as observed in AMORIS, four studies corroborated these findings [14, 43, 48, 49, 51].

Where are we today?

Dyslipidaemia is closely linked to obesity, another emerging risk factor for several cancers [52]. This implies that despite the suggested mechanisms, abnormal lipid metabolism may be a proxy of other lifestyle-related factors underlying carcinogenesis. Nevertheless, there is evidence suggesting that statins, a class of lipid-lowering drug, may suppress cell proliferation and increase apoptosis by inhibiting the action of the enzyme hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase [53–55], further indicating the involvement of lipids in carcinogenesis. The inverse association between ApoA-I and cancer as found in our study was potentially related to not only inflammation [56], but other lifestyle factors such as body mass index (BMI), cigarette smoking, alcohol intake, diabetes, or hypertension influencing the circulating levels of ApoA-I. This lipid biomarker has been shown to be predictive of cardiovascular risk [4, 57] and it is thus possible that the oetiological pathway between lipid profiles and atherosclerosis is different from the pathway between lipid profiles and cancer. The strong association between the lipid metabolism and cardiovascular disease also indicates a potential competing risk situation [58], where individuals at risk of cancer may die of cardiovascular disease before being diagnosed with cancer. This urges further studies to address the issue especially when assessing serum lipids in relation to cancer.

Glucose metabolism

Selected biomarkers

Disruptions in the glucose metabolism, which encompass an array of metabolic abnormalities such as diabetes, have been linked to chronic diseases including cancer [59]. Serum **glucose** is the most commonly measured marker of the glucose metabolism, representing current levels of glucose in the circulation. **Fructosamine** is another commonly used marker and reflects the average level of serum glucose in the previous 10–14 days [60]. Insulin, with elevated levels marking the initial stage of impaired glucose metabolism, has been suggested to be involved in carcinogenesis through its growth-promoting effects on cells [61]. Similar mutagenic effects have been suggested for a closely linked marker, insulin-like growth factor I (IGF-I) [62]. Additionally, serum glucose may directly affect cancer through generation of Advanced Glycation End-products (AGE), which leads to chronic inflammation [63]. Fructosamine, which represents all glycated serum proteins, may therefore also be involved in this mechanism. The role of impaired glucose metabolism in cancer development and survival has been suggested [64], for instance, Hammarsten *et al* showed in a prospective study of 320 prostate cancer patients that men who died of clinical prostate cancer during follow-up had a higher prevalence of type 2 diabetes ($P < 0.035$) and higher levels of fasting plasma insulin ($P = 0.004$) [65]. These results indicated that insulin levels could be used as markers of prostate cancer prognosis and tumour aggressiveness, regardless of the patient's prostate cancer stage, cancer grade, and PSA level. Data from another prospective cohort in Sweden also

Table 1. Epidemiological studies on lipid metabolism and cancer.

Publication	Study population	Study design	No. Of subjects, follow-up	Exposure	Outcome	Main results	Adjustments
Haggstrom, H. 2012 [39]	Me-Can cohort	Prospective cohort	289,866 men included.	Smoking status, BMI, blood pressure, glucose, cholesterol, and TG.	PCa risk	High levels of triglycerides were associated with a decreased risk of pca top quintile RR 1.24 (1.06–1.45) bottom quintile 0.88 (0.74–1.04).	Smoking, BMI.
Jacobs, E.J.2012 [40]	Cancer prevention study II nutrition cohort	Cohort.	236 cases and 236 matched controls.	TC, LDL cholesterol, HDL cholesterol, non-HDL cholesterol. (non-fasting).	PCa risk	Neither total, LDL, nor HDL cholesterol concentrations were associated with risk of pca. OR 0.93 (95% CI 0.76–1.14) for total cholesterol and 0.97 (95% CI 0.82–1.16)	Age, race, blood draw date, physical activity, use of cholesterol-lowering drugs, and history of heart attack.
His, M 2014. [49]	Supplementation en vitamines et mineraux antioxydants study	Cohort	7557 subjects	TC, LDL cholesterol, HDL cholesterol, TG, ApoA1, apob	Breast cancer and PCa risk	TC was inversely associated with overall (HR = 0.91 95% CI 0.82–1.00) and breast (HR = 0.83 95% CI 0.69–0.99) cancer risk. HDL-c was also inversely associated with overall (HR = 0.61 95% CI 0.46–0.82) and breast (HR = 0.48 95% CI 0.28–0.83) cancer risk. Consistently apoa1 was inversely associated with overall (HR = 0.56 95% CI 0.39–0.82) and breast (HR = 0.36 95% CI 0.18–0.73) cancer risk.	Age, intervention group, number of dietary records, alcohol intake per day, physical activity. Smoking status, educational level, height, BMI, family history of bca, menopausal status at baseline, TG-lowering drugs antihypertensive drugs, energy intake per day and glycaemia. Ratio models adjusted for TG and TC.
Wu, Q. 2012 [48]	Hospital PUMCH patient information database	Case-control	210 pancreatic adenocarcinoma, 630 healthy controls	TC, LDL cholesterol, HDL cholesterol, TG, ApoA1, apob, fasting blood glucose.	Pancreatic adenocarcinoma risk	TC (OR–1.793 95% 1.067–3.013) and ApoA (OR = 36.065 95% 15.547–83.663) were significantly related to pancreatic adenocarcinoma.	Age and sex.
Agnoli, C. 2014 [41]	Colorectal cancer cases	Cohort	1134 participants 850 in randomly selected cohort and 286 colorectar cancer cases	TC, LDL cholesterol, HDL cholesterol, TG. (Fasting)	Colorectal cancer risk	Highest tertiles of total (HR = 1.66 95% 1.12–2.45) and LDL cholesterol (HR1.87 95% CI 1.27–2.76) were associated with increased colorectal cancer risk.	Age, gender, BMI, smoking, total physical activity, alcohol consumption, dietary red meat, dietary fiber, and dietary calcium.
Jiang, R. 2014 [51]	Cancer registry	Cohort	807 patients.	TC, LDL cholesterol, HDL cholesterol, TG, ApoA1, ApoB,	Nasopharyngeal carcinoma survival	ApoA-I levels (HR = 0.64 95% CI 0.52–0.80) were associated with a favourable OS.	Adjustment for clinical characteristics and other serum lipids and lipoproteins
Kim, H.S.2013 [42]		Cohort	14932	BMI, H.pylori, TC, LDL-c, HDL-c, TG	Prevalence and risk factors of colorectal cancer	Predictor of colorectal cancer was hypertriglyceridemia (OR = 1.267 95% CI 1.065–1.508)	–

Table 1. Continued.

Shafique, K. 2012 [38]	Midspan studies	Prospective cohort study	12,926 men (650 cases)	Baseline cholesterol	Incidence of pca and prognosis	Baseline plasma cholesterol was associated with hazard of high grade PCa incidence (n = 119).	Association remained significant after adjustment for body mass index, smoking and socioeconomic status
Kitahara *et al* 2011 [35]	Korean adults enrolled in the National Health Insurance Corporation	Cohort	53,944 men and 24,475 women	TC (fasting)	Cervix, breast, colon, lung, pancreas, bladder, kidney, oesophagus, gall bladder, liver, rectal, prostate cancer risk	TC (≥ 240 mg/dL) was associated with PCa (HR 1.24; 95% CI, 1.07 –1.44; P = 001) and colon cancer (HR, 1.12; 95% CI, 1.00–1.25; P = 05) in men. Breast cancer (HR, 1.17; 95% CI, 1.03 –1.33; P trend = 03). Total cholesterol was inversely associated with all-cancer incidence in both men (HR, 0.84; 95% CI, 0.81–0.86; P < 001) and women (HR, 0.91; 95% CI, 0.87–0.95; P < .001).	Adjustments for cigarette smoking, alcohol consumption, BMI, physical activity, hypertension and fasting serum glucose .
Mondul *et al*, 2011 [37]	ATBC Study	Cohort	2041	TC, HDL (fasting)	PCa risk	Men with higher serum TC were at increased risk of overall (≥ 240 versus <200 mg/dl: HR = 1.22, 95% CI 1.03–1.44, ptrend = 0.01) and advanced (≥240 versus<200 mg/dL: HR = 1.85, 95% CI 1.13–3.03, p-trend = 0.05) prostate cancer	Adjusted for serum α-tocopherol, family history of prostate cancer, education level, and urban residence, other cholesterol type, smoking habits, BMI, marital status; total energy, total fat, fruit, vegetable, red meat, alcohol, dietary retinol, vitamin D, calcium intake. Subgroup analyses were conducted stratifying by follow-up time (<ten years, >ten years).
Kok *et al*, 2011 [36]	Nijmegen Biomedical Study	Cohort	2842	TG, TC, HDL, LDL	PCa risk	Higher total and higher LDL cholesterol were significantly associated with an increased risk of prostate cancer HR 1.39 (95% CI 1.03–1.88) and 1.42 (95% CI 1.00–2.02), respectively. Similar results were observed for aggressive prostate cancer, whereas for non-aggressive prostate cancer a significant association with HDL cholesterol was found HR 4.28, 95% CI 1.17–5.67.	Adjusted for age, body mass index and history of diabetes mellitus

Table 1. Continued.

Agnoli *et al*, 2010 [47]	Cancer registry	Cohort	163	TG, HDL	Breast cancer risk	Metabolic syndrome associated with breast cancer risk (rate ratio 1.58 [95% confidence interval 1.07–2.33]). Low serum HDL-cholesterol and high triglycerides were significantly associated with increased risk	Adjusted for matching variables and for: age, age at menarche, years from menopause, number of full-term pregnancies, age at first birth, oral contraceptives, hormone therapy, years of education, history of breast cancer in first degree relatives, breastfeeding, smoking, and alcohol consumption.
Bjorge *et al*, 2011 [50]	Me-Can study	Cohort	644	TG, TC (fasting and non-fasting)	Ovarian cancer	–	Year of birth, age at measurement, smoking and quintile levels of BMI
Van Duijnhoven *et al*, 2011 [43]	EPIC study	Nested case-control (EPIC)	1238	TG, TC, HDL, LDL, Apo A-1, Apo B (NS)	Colorectal cancer risk	HDL and apoA were inversely associated with the risk of colon cancer (RR for 1 SD increase of 16.6 mg/dl in HDL and 32.0 mg/dl in apoA of 0.78 (95% CI 0.68–0.89) and 0.82 (95% CI 0.72-0.94), respectively.	Height, weight, smoking habits, physical activity, education, consumption of fruit, vegetables, meat, fish and alcohol, intake of fibre, energy from fat and energy from non-fat
Hu *et al*, 2011 [43]	Cancer registry	Case-control	397	TG, HDL (fasting)	Colorectal cancer risk	TGs associated with cancer risk : HR for ≥150mg/dl vs <150mg/dL: 1.18; 95% CI: 0.9–1.51. HDL (-): HR for < 40mg/dL versus ≥40mg/dL (men) or <50 mg/dL versus ≥ 50mg/dL (women): 0.94; 95% CI: 0.71–1.24.	Age, sex, smoking, drinking, past history of adenoma, other components of metabolic syndrome.
Aleksandrova *et al* 2011 [45]	EPIC study	Nested case-control (EPIC)	689	TG, HDL, (fasting and non-fasting)	Colon, rectal, cancer risk	Reduced HDL associated with colon cancer risk RR for ≤ 40 mg/dL versus > 40mg/dL in men and ≤ 50mg/dL versus > 50mg/dL in women: 1.36; 95% CI: 1.04–1.77.	Smoking status, education, alcohol consumption, physical activity, fiber intake, consumption of fruits and vegetables, red and processed meat, fish, and shellfish.
Stocks *et al*, 2011 [46]	Me-Can study	Cohort	2834 men, 1861 women	TG, TC (fasting and non-fasting	Colorectal cancer risk	TGs were found to be positively associated with cancer risk RR for fifth versus first quintile: 1.65; 95% CI: 1.27–2.13 (men). RR for fifth versus first quintile: 1.42; 95% CI: 1.09–1.85 (women).).	Smoking, five categories of birth year, age at measurement and quintiles of BMI

suggested that insulin resistance related factors might be important for tumour progression [66]. With regards to breast cancer, two genetic variations (MNTR1a and 1b genes) have been shown to be associated both with cancer susceptibility and perturbed expression of insulin and glucose [67].

Findings in AMORIS

Apart from the interplay between glucose, triglycerides, total cholesterol, we also investigated possible associations between glucose levels and risk of breast, endometrial, and ovarian cancer in a cohort of more than 230,000 women [7, 8, 21]. Our results indicated that glucose levels below diagnostic threshold for diabetes increased the risk of endometrial and postmenopausal breast cancer. Most recently, we investigated repeated measurements of glucose, and fructosamine in relation to cancer risk and found highest cancer risks for those in the highest tertile of glucose and lowest tertile of fructosamine [16].

New epidemiological findings in the literature

The more recent literature provides further epidemiological evidence on how the glucose metabolism play a role in the risk of a number of cancers such as colon, liver, and endometrial cancers [68–72] (Table 2). Interestingly, one study on thyroid cancer found a positive association for serum glucose in men and an inverse association in women [73]. This might imply a different role of the glucose metabolism in endocrine-related cancers. However, studies on the role of serum glucose concentrations and breast cancer risk were inconclusive [74]. No new findings have been reported for the link between fructosamine and risk of cancer.

Where are we today?

Common key players in impaired glucose metabolism and cancer may indicate that both share an underlying mechanism rather than any causal role of serum glucose in carcinogenesis [75]. However, a protective effect against cancer has been suggested for metformin, one of the main medications to lower blood glucose [76–78], which supports the role of the glucose metabolism. In addition to glucose-lowering effects, metformin also possesses a direct anti-tumour effect by inhibiting protein synthesis and cell proliferation [79]. Another issue to be addressed when assessing the glucose metabolism in relation to cancer is turnover times for the serum markers. Fructosamine and HbA1c, which remain in the circulation for a longer duration than serum glucose, may provide more accurate representation of individual glycaemic status. The variability of serum glucose may also be accounted for by using multiple measurements as performed in one of our studies [16], either as a cumulative average or time-varying covariates [80]. Future studies should also consider the role of glucose metabolism markers in other chronic diseases, which may distort its association with cancer.

Immune system

Selected biomarkers

The role of the immune system in carcinogenesis was first shown by an observation of cancer occurring in chronic inflammation [81]. It is thought that inflammation is capable of triggering both tumour initiation and promotion through the formation of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) [82]. **C-reactive protein** (CRP) is one of the most investigated markers of inflammation in the context of cancer detection and prognosis. Higher levels of post-diagnosis CRP have been linked with worse survival rates in various malignancies [83–85]. In addition to CRP, **albumin, haptoglobin,** and **leukocytes** are other commonly used markers of inflammation. Albumin is an acute-phase protein involved in blood volume regulation and transportation of molecules of low water solubility such as lipid soluble hormones and calcium. Together with leukocytes, albumin has been studied as a marker of systemic inflammation in the context of cancer survival and so far results have shown that low levels of albumin and high levels of leukocytes are associated with worse cancer prognosis [86]. **Haptoglobin** is a positive acute-phase protein and its plasma levels increase during inflammatory processes such as infection, extreme stress, burns, major crush injury, or allergy. The full scope of the biological function of haptoglobin is not yet defined, however

Table 2. Epidemiological studies on glucose metabolism and cancer.

Publication	Study population	Study design	No. of subjects, follow-up	Exposure	Outcome	Main results	Adjustments
Parekh, 2013 [68]	The Framingham Offspring Cohort, USA, men and women, age 20+ years	Cohort	3707 without cancer, duration 37 years	Fasting serum glucose	Obesity-related cancers	HR: 1.57 (95% CI: 1.17–2.11) for fasting glucose >110 mg/dL versus lower (measured 20+ years prior to censoring time)	Adjusted for age, sex, alcohol, smoking, and BMI. Obesity-related cancers were defined as cancers of the gastrointestinal tract, reticulo-endothelial systems, female reproductive tracts, genitourinary organs, and the thyroid gland. Similar increased risk for colon cancer
Boyle, 2013 [74]	USA, Austria, Sweden, Korea, Italy	Meta-analysis	Six cohort, three case control, one case cohort studies	Serum glucose	Breast cancer	Summary RR: 1.11 (95 % CI: 1.00–1.23)	I^2: 0 %
Friedenrich, 2012 [69]	Canada, women, mean age 59 (cases) and 59 (controls)	Case control	514 cases, 962 controls	Serum glucose	Endometrial cancer	OR: 1.26 (95% CI: 1.11–1.43) for every unit increase	Matched on age groups. Adjusted for age
Ulmer, 2012 [70]	Metabolic syndrome and cancer project (Me-Can), Austria, Norway, Sweden, women, mean age 44.1 years	Cohort	288274 without cancer, mean FU 11.3 years	Serum glucose	Cervical cancer	HR: 0.62 (95% CI: 0.20–1.96) for the highest versus lowest quintile	Stratified by centre, sex, and year of birth. Adjusted for age, smoking
Borena, 2011 [71]	Metabolic syndrome and cancer project (Me-Can), Austria, Norway, Sweden, men and women, mean age 43.9 (men) and 44.1 (women)	Cohort	406364 without cancer, mean FU 12.8 years (men) and 11.3 years (women)	Serum glucose	Liver cancer (primary)	RR: 2.38 (95% CI: 1.76–3.14) for every log unit increase	Stratified by cohort, birth year, and sex. Adjusted for age, smoking
Almquist, 2011 [73]	Metabolic syndrome and cancer project (Me-Can), Austria, Norway, Sweden, men and women, mean age 43.9 (men) and 44.1 (women)	Cohort	578700 without cancer, mean FU not specified	Serum glucose	Thyroid cancer	RR: 9.24 (95% CI: 1.46–59.6) in men, 0.16 (0.01–0.69) in women, for the highest versus lowest quintile	Stratified by cohort, age. Adjusted for BMI, smoking, age
Johansen, 2010 [72]	Metabolic syndrome and cancer project (Me-Can), Austria, Norway, Sweden, men and women, mean age 43.9 (men) and 44.1 (women)	Cohort	577315 without cancer, mean FU 12.8 years (men) and 12.8 years (female)	Serum glucose	Pancreatic cancer	RR: 2.05 (95% CI: 0.84–4.94) for the highest versus lowest quintile	Stratified by cohort, birth year. Adjusted for BMI, smoking, age

experimental studies have hypothesised that haptoglobin polymorphisms may contribute to increased oxidative stress and low-grade chronic inflammation [87, 88]. There is also observational evidence indicating that allergy (measured by **Immunoglobulin E** (IgE)), which is highly linked to inflammation, is associated with higher risk of solid tumours such as breast, prostate, and colorectum [9].

Findings in AMORIS

We have studied different immunological markers in relation to cancer risk [9, 13, 22]. One study was of particular interest, because it replicated the findings for one measurement of CRP and leukocytes with three repeated measurements [13]. When looking into specific major cancers including prostate, breast, lung, gastrointestinal, bladder, cervix. and skin cancer, a positive association was only seen for lung cancer. The lack of association between inflammatory markers and specific cancer risk was further shown when we investigated serum CRP, leukocytes, albumin, and haptoglobin in relation to prostate cancer [22]. We also assessed the association between total serum levels of IgE and cancer risk in 24,820 persons and found a weak inverse association between quartiles of IgE and cancer risk [9].

New epidemiological findings in the literature

A consistent association between serum CRP and cancer risk is corroborated by more recent findings (Table 3), as shown by a meta-analysis of 11 studies in Western populations showing an increased cancer risk for higher levels of CRP [89]. Similar findings were reported in Asian populations [90]. Results for specific cancers remain conflicting except for lung cancer, where a positive association with CRP and leukocytes has been reported. This is consistent with our findings seen in the AMORIS database [89, 91, 92]. Some evidence, although weaker, has been reported for colorectal, breast, ovarian. and liver cancer [93–97], whereas no association has been found for prostate and pancreatic cancers [98–100]. Regarding serum IgE, most observational studies confirmed an inverse association with risk of developing brain cancer, particularly glioma [101–104]. To date, little evidence exists for association with any other cancers.

Where are we today?

Although biological studies consistently link inflammation to carcinogenesis [105], the role of common serum inflammatory markers in predicting cancer risk still remains unclear. This may be partly because of the wide spectrum of inflammation, which is also an essential part of many pathologic conditions such as cancer and cardiovascular disease. The non-specificity of such cancer markers may explain the lack of associations found in observational studies, urging future studies to deploy novel methods to increase sensitivity of cancer prediction using these markers. Another possible explanation is the genetic variation of these markers, instead of their quantitative protein expression, that influences cancer development. This is supported by two recent studies suggesting different risk of colorectal cancer conferred by CRP polymorphisms [106, 107]. Additionally, these markers are usually analysed separately and a combined analysis may provide a better approximation with respect to early cancer detection, as it has shown in the case when combining scores of CRP with IL-8 [108] or haptoglobin with serum amyloid A (SAA) [109] in predicting lung cancer risk, and the ratio of reactive oxygen metabolites and CRP for colorectal cancer [110].

Liver metabolism

Selected biomarkers

Gamma-glutamyl transferase (GGT), is a central enzyme in the glutathione (GSH) metabolism, a ubiquitous antioxidant thiol, and plays an important role in maintaining tissue oxidant/antioxidant balance, cellular defence, proliferation, and protection against further oxidative stress [111]. The latter may explain its potential role in carcinogenesis, in addition to its links with type 2 diabetes, cardiovascular, and chronic kidney disease [112–115]. Elevated levels of GGT have been associated with poorer endometrial cancer prognosis, increased risk of progression of high-grade cervical dysplasia to invasive carcinoma [116], increased risk of breast cancer amongst premenopausal women [117], increased risk of cancer in men [118], increased risk of liver cancer [119] and it has been reported to play an independent role in the prediction of overall survival (OS) in metastatic colorectal carcinoma [120].

Table 3. Epidemiological studies on Immune system and cancer.

Publication	Study population	Study design	No. of subjects, follow-up	Exposure	Outcome	Main results	Adjustments
Guo, 2013 [89]	USA, UK, Denmark, Sweden	Meta-analysis	194796 total participants, 11459 cancer	CRP	Overall cancer	Summary RR: 1.11 (95% CI: 1.03–1.18)	$P_{heterogeneity} < 0.0001$, $I^2 = 70\%$
Lee, 2011 [90]	South Korea, men and women, mean age 55 in cases and 47 in non cases	Cross-sectional	80781 without cancer, mean FU	CRP	Overall cancer	OR: 1.94 (95% CI: 1.51-2.51) for CRP >3 versus< 1 mg/L)	Adjusted for age, sex, BMI, diabetes, hypertension, dyslipidemia, smoking, alcohol consumption, exercise, aspirin use, education level, and income
Xu, 2013 [92]	China, men and women, age 36–68 years	Case control	96 cases, 124 controls	CRP	Lung cancer	OR: 2.11 (95 % CI, 1.66–2.91) for highest quartile versus lowest	Adjusted for smoking, gender, height, age, race, BMI, education, occupation, and living place
Dossus, 2014 [93]	The E3N prospective cohort, France, women, born between 1925–1950	Case control	549 cases, 1040 controls	CRP	Postmenopausal breast cancer	OR: 1.24 (96% CI: 0.92–1.66) for CRP 2.5–10 mg/L versus< 1.5 mg/L	Adjusted for matching variables: age at blood collection, menopausal status at blood collection, year of blood collection, centre of collection, and age at menopause
Toriola, 2013 [94]	Women's Health Initiative Observational Study (WHI-OS), USA, women, age 50–79 years	Case control	988 cases, 988 controls	CRP	Colorectal cancer	OR: 1.30 (0.93–1.82) for highest quintile versus lowest	Matched on age, race, centre, date of blood-draw, baseline hysterectomy status. Adjusted for age, BMI, hormone replacement therapy, previous colonoscopy, pack-years of smoking use
Toriola, 2013 [100]	the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD), Finland, men, age 42–60 years	Cohort	203 free from cancer, mean FU 24 years	CRP	Prostate cancer	1.08 (95% CI: 0.74–1.60) for highest tertile versus lowest	Adjusted for age, examination year, socioeconomic status, alcohol consumption, energy intake, cardio-respiratory fitness, BMI and smoking
Toriola, 2011 [97]	The Finnish Maternity Cohort (FMC), Finland, women, mean age 28.6 (cases) and 28.7 (controls)	Case control	91 cases, 115 controls	CRP	Ovarian cancer	OR: 1.62 (0.93–2.83) for highest tertile versus lowest	Adjusted for age
Trabert, 2014 [95]	The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, USA, women, age 55–74 years	Case control	149 cases, 149 controls	CRP	Ovarian cancer	OR: 2.04 (1.06–3.93) for highest tertile versus lowest	Matched on age, race, study centre, time and date of blood collection. Adjusted for BMI, smoking, parity, duration of oral contraceptive use, and duration of menopausal hormone therapy use

Table 3. Continued.

Aleksandrova, 2014 [96]	The European Prospective Investigation into Cancer and Nutrition (EPIC), Europe, men and women, 35–75 years	Case control	125 cases, 250 controls	CRP	Hepatocellular carcinoma	RR: 1.22 (1.02–1.46) per doubling of serum level	Matched on study center, sex, age, date of blood collection, fasting status, and time of blood collection. Women were additionally matched on menopausal status and exogenous hormone use. Adjusted for education, smoking, alcohol, diabetes, coffee, HBsAg/anti-HCV, BMI and waist to height ratio (WHtR)
Bao, 2013 [98]	The Health Professionals Follow-up Study (HPFS), the Nurses' Health Study (NHS) the Physicians' Health Study I (PHS I), the Women's Health Initiative (WHI), the Women's Health Study (WHS), USA,	Case control	491 cases, 1137 controls	CRP	Pancreatic cancer	OR: 0.99 (0.98–1.01) for every unit increase	Matched on year of birth, prospective cohort (which concurrently matched on sex), smoking status, fasting status, and month of blood draw. Adjusted for race, history of diabetes, BMI, physical activity, current vitamin use, levels of vitamin D and C-peptide
Grote, 2012 [99]	The European Prospective Investigation into Cancer and Nutrition (EPIC), Europe, men and women, 35–75 years	Case control	455 cases, 455 controls	CRP	Pancreatic cancer	OR: 1.01 (0.92–1.11) per doubling of serum level	Matched on recruitment centre, sex, age, date at entry, time between bloodsampling and last consumption of foods and drinks, hormone use. Adjusted for smoking and BMI
Calboli, 2011 [101]	The Health Professionals Follow-up Study (HPFS), the Nurses' Health Study (NHS), the Physicians' Health Study (PHS), the Women's Health Study (WHS), USA,	Case control	169 cases, 520 controls	Total IgE	Glioma	OR: 0.97 (0.88–1.07) for every unit increase	Matched on year of birth, cohort (which automatically matches the sex), month of blood collection, and ethnic background.
Schlehofer, 2011 [102]	The European Prospective Investigation into Cancer and Nutrition (EPIC), Europe,	Case control	696 cases, 1188 controls	Allergen-specific IgE	Glioma	OR: 0.73 (0.51–1.06) for positive versus negative	Matched on study centre, sex, date of birth, age, date and time of blood collection , length of follow-up. Adjusted for education and smoking. Similar non statistically significant results for meningioma and schwannoma
Schwartzbaum, 2012 [103]	The Janus Serum Bank cohort, Norway, men and women, age 35–49 years	Case control	594 cases, 1177 cases	Allergen-specific IgE	Glioma	OR: 0.95 (0.75–1.22) for positive versus negative	Matched on two-year age interval, sex, and date of blood collection
Wiemels, 2011 [104]	USA, men and women, age 20–79 years	Case control	61 cases, 192 controls	Total IgE	Meningioma	OR: 0.85 (95% CI: 0.75–0.98	Matched onfive-year age interval, sex, and state of residence. Adjusted for sex, race, smoking, age, education

Findings in AMORIS

We have investigated GGT serum levels in relation to cancer risk in 545,460 persons and found evidence of associations between elevated GGT and risk of developing different cancers. The strength of this association varied by levels of glucose which may suggest that hyperglycaemia can result in oxidative stress which in turn initiate damaging pathways of carcinogenesis [19].

New epidemiological findings in the literature

Since the last AMORIS publication, several studies have analysed the association between GGT and cancer risk and prognosis [121–128] (Table 4). All studies are in agreement with our findings in AMORIS and show that high levels of GGT are an indicator of elevated cancer risk and poor disease prognosis. Three studies showed that high pre-therapeutic levels of GGT are associated with advanced tumour stage and serve as an independent prognostic marker of poor prognosis in gynaecological cancers [122, 125, 126]. A case-cohort study in Taiwanese men showed that high levels of GGT were associated with risk of all-cause death, all cancer, and hepatocellular carcinoma (HCC) mortality [124]. Furthermore, another study analysing GGT and HCC prognosis showed that high levels of pre-treatment GGT were associated with reduced OS rates, when compared to those with normal pre-treatment GGT levels [121]. In addition, elevation of serum GGT levels was found to be an indicator of aggressive intrahepatic cholangiocarcinoma behaviours and a predictor of poor clinical outcomes [127]. Interestingly, one study in Japanese adults found that GGT was only a predictor of cancer risk for alcohol-related cancers in current drinkers [123]. GGT has also been reported to play an independent role in the prediction of OS in metastatic colorectal carcinoma [120].

Finally, a meta-analysis by Long *et al* concluded that GGT predicts cardiovascular and cancer mortality [129], whereas Kunustor *et al* in their meta-analyses showed that baseline levels of GGT are positive independent predictors of overall cancer risk as well as for all-cause mortality [130, 131].

Where are we today?

Overall epidemiological evidence shows that high levels of GGT are associated with cancer risk and many experimental studies have intended to explain this link suggesting different biological mechanisms [132–136]. These pathways have been demonstrated for cancer specific sites which may be explained by the high variability present in cancer cells together with the effect of other factors, such as environment, drugs, and diet that could modify cancer cells phenotype including GGT expression [137].

Iron metabolism

Selected biomarkers

The iron metabolism is another pathway potentially linked with carcinogenesis. Iron plays a fundamental role in important biological processes in eukaryotic cells such as oxygen transport, cellular respiration, and redox reactions; consequently iron homeostasis is precisely regulated. Most **circulating iron** is bound to transferrin; the rest of iron is either serum-free iron or iron stored in cells bound to ferritin. **Total iron-binding capacity (TIBC)** measures the ability of plasma proteins to bind iron and reflects the fraction of transferring- free places to bound iron, meaning that low values of TIBC evidence transferrin saturation (TSAT) and consequently high iron stores in cells.

Different mechanisms of iron involvement in carcinogenesis have been suggested, including oxidative DNA damage by iron-catalysed free radical production, alterations in gene expression consistent with increased iron requirements in proliferating cells, as well as decreased immune surveillance against cancer [138]. Excess iron has been shown to promote protein and genomic alterations mirrored in human cancers [139] and this may occur via iron-induced persistent oxidative stress [139]. Moreover, iron sequestration machinery is activated by inflammatory processes associated with chronic diseases such as breast cancer for which cancer-associated anaemia is being broadly studied [140].

Table 4. Epidemiological studies on liver metabolisms and cancer.

Publication	Study population	Study design	No. of subjects, follow-up	Exposure	Outcome	Main results	Adjustments
Zhang *et al* 2011 [121]	Cancer registry	Cohort	277	GGT	Hepatocellular carcinoma prognosis	The one-year and three-year OS rates were 71.6 and 38.5% in patients with normal GGT and 48.8 and 16.9% in patients with high GGT (P = 0.002).	–
Yin *et al* 2013 [127]	Cancer registry	Cohort	411	GGT	Intrahepatic cholangiocarcinoma prognosis	GGT was an independent predictor of a poor prognosis (hazard ratio =2.36, 95% confidence interval: 1.67–3.34, P = 0.001)	–
Tsuboya *et al* 2012 [123]	Ohsaki Cohort Study	Cohort	15 031	GGT	Overall cancer incidence	Highest quartile (GGT ≥31.0 IU/mL), the multivariate HR for any cancer was 1.28 (95% CI, 1.08–1.53; P for trend, <0.001), the HR for colorectal cancer was significantly greater than unity. This positive trend was observed only in current drinkers	Adjusted for age sex, drinking habit, self-reported history of liver disease, smoking habit body mass index, education, exercise.
Seebacher *et al* 2012 [122]	Multicenter database	Multicenter trial	874	GGT	Endometrial Cancer prognosis	Elevated serum GGT levels (P = 0.03 and P = 0.005), tumour stage (P < 0.001 and P < 0.001), grade (P < 0.001 and P = 0.02) and age (P < 0.001 and P < 0.001) were independently associated with progression-free survival in univariate and multivariable survival analyses	Patients were stratified in GGT risk groups
Hofbauer *et al* 2014 [128]	Cancer registry	Cohort	921	GGT	Renal cell carcinoma prognosis	Gamma-glutamyltransferase levels increased with advancing T (P < 0.001), N (P¼ 0.006) and M stages (Po0.001), higher grades (P < 0.001), and presence of tumour necrosis (Po0.001). An increase of GGT by 10UI 1 was associated with an increase in the risk of death from RCC by 4% (HR 1.04, P < 0.001).	Adjusted for T stage, N stage, M stage, Fuhrman grade, necrosis histologic subtype.
Hernaez *et al* 2013 [124]	MJ Health Study	Case-Cohort	3961	GGT	Hepatocellular carcinoma mortality	High levels of GGT were associated with cancer mortality (HR1.8–2.8) and HCC mortality (HR 5.5–36.1).	Adjusted for age at baseline, body mass index, physical activity, smoking and alcohol use, education, systolic and diastolic blood pressure, total cholesterol, HDL, C-reactive protein HBsAg

Table 4. Continued.

He *et al* 2013 [120]	Cancer registry	Cohort	239	GGT	Colorectal Carcinoma prognosis	GGT ($P < 0.001$) statistically significant prognostic factor of overall survival validated as independent predictor. On univariate analysis, GGT ($P < 0.001$) statistically significant predictive factor of progression-free survival (PFS) in patients having first-line chemotherapy	–
Grimm *et al* 2013 [125]	Cancer registry	Multicenter study	634	GGT	Ovarian cancer prognosis	High GGT serum levels were associated with advanced FIGO stage ($P < 0.001$) and with worse overall survival in univariate ($P < 0.001$) and multivariable analysis ($P = 0.02$, HR 1.2 (1.1–1.5)	Adjusted for continuous GGT values and survival
Edlinger *et al* 2013 [126]	Vorarlberg Health Monitoring and Promotion Programme	Sub-Cohort	318	GGT	Endometrial cancer prognosis	GGT associated with cancer-related mortality (HR = 3.35, 95% CI 1.12–10.03)	Adjusted for age, tumour-staging (FIGO) and histology, together with the examination year, body mass index, hypertension, triglycerides, total cholesterol, glucose.

Findings in AMORIS

Using a cohort of 220,642 participants with baseline measurements of serum iron, TIBC, and CRP, we found a positive association between TIBC (i.e. low TSAT) and the risk of overall and in particular colon cancer [5]. Serum iron, on the other hand, did not correlate with overall cancer risk, although a positive association with postmenopausal breast cancer was shown. These observations thus support a role of iron metabolism in relation to specific cancer risk.

New epidemiological findings in the literature

Only one recently published study focused on serum iron as a marker of the iron metabolism in the context of cancer risk. This cohort study of 309,443 men and women in Taiwan reported an increased risk of cancer in individuals with high serum iron [141]. Specific cancer analysis showed an increased risk of breast cancer for serum iron ≥140 μg/dL—hazard ratio (HR): 1.31 95%, confidence interval (CI): 1.01–1.70—compared to lower levels, which is similar to our findings for postmenopausal breast cancer. Other recent studies measured iron based on dietary intake subclassified as dietary heme iron, supplemental iron, and dietary intake of meat [142–145]. Dietary iron was assessed mainly using food frequency questionnaires and heme iron intake was usually determined indirectly by calculating a type-specific percentage of the total iron content in meat [144, 145]. Furthermore, a broad meta-analysis examining different cancer types in association with serum iron markers and dietary iron markers, found a negative association between cancer risk and levels of iron storage biomarkers, mostly with serum ferritin. Moreover, authors reported that a higher intake of heme iron showed a tendency towards a positive association with cancer risk [146]. Similar conclusions for dietary markers were obtained in a colorectal cancer meta-analysis, suggesting a significant positive association of heme iron intake and risk of colorectal cancer [147].

Where are we today?

Iron homeostasis is closely linked to anaemia, which impairs many physiological processes [148]. Considering the association between anaemia and mortality [149], it is possible that the positive association between serum iron and risk of cancer emerges as a consequence of other fatal diseases in persons with low levels of iron, thus removing them from the population at risk of developing cancer. Future research should address risks associated with different types of anaemia in addition to serum components of iron metabolism when assessing their link to cancer susceptibility.

Bone metabolism

Selected biomarkers

Components of bone metabolism have been indicated to be involved in carcinogenesis. Since calcium homeostasis is mainly influenced by vitamin D and parathyroid hormone instead of dietary calcium [150], the use of serum **calcium** could be useful in investigating the aetiology of cancer. Ionised serum calcium level is a direct measure of the amount of metabolically active serum calcium but is not routinely measured [151]. **Correction of total calcium levels based on serum albumin** is therefore used to obtain an estimate of the free ionised calcium level, since almost half of serum calcium is in protein-bound form and alteration of serum albumin may affect levels of free ionised calcium [150, 151]. **Inorganic phosphate** (Pi) is another dietary constituent well-known for its role in skeletal mineralisation, and normal levels of Pi are essential to maintain normal cellular function [152]. As a result, it has been suggested that Pi may act as an active regulator of growth rather than a merely compulsory element in cellular homeostasis. A particular link between calcium and gastrointestinal cancer has been suggested, since dietary calcium may activate calcium receptor and bind bile acids in gastrointestinal tract, in addition to the role of serum calcium in cellular metabolism [153, 154]. Recent studies also indicated that inorganic phosphate might be implicated in carcinogenesis, as high-inorganic phosphate diet has been linked to an increased development of lung and skin cancers [155, 156]. Abnormal levels of inorganic phosphate are thought to affect carcinogenesis by amplification of Akt signalling and 5′ cap eukaryotic dependent translation [157, 158].

Findings in AMORIS

We investigated serum calcium in relation to risk of prostate and gastrointestinal cancer, and serum inorganic phosphate in relation to risk of overall and site-specific cancers [5, 17, 18, 20]. We found a weak negative association between calcium and prostate cancer, which was likely explained by a strong association between calcium and all-cause mortality. For gastrointestinal cancer, higher risks of oesophageal and colorectal cancer were linked to higher levels of albumin-corrected calcium in women, indicating the importance of calcium correction based on albumin levels. In men, a similar but weaker association was found. The study focusing on inorganic phosphate showed a positive association with risk of overall cancer in men, but an inverse association in women.

New epidemiological findings in the literature

In support of the above findings, another Swedish-based study showed a positive trend between levels of albumin-adjusted calcium and risk of prostate cancer in men [159] (Table 5). Similar findings with total and ionised serum calcium were reported when prostate cancer death was used as a surrogate outcome [160]. Nonetheless, an inverse association was observed in an Asian study [161]. No new studies have been published investigating the association between serum inorganic phosphate and risk of cancer.

Where are we today?

In clinical studies, the potential chemopreventive effects of calcium in cancer, particularly colorectal cancer, remain conflicting [162]. A recent dose-response meta-analysis showed an inverse association between dietary calcium, calcium supplementation, and risk of colorectal cancer [163]. However, the role of serum levels of calcium as well as its counterpart, serum inorganic phosphate, in relation to cancer prediction remains elusive. As bone metabolism is tightly regulated, abnormalities in calcium and phosphate levels may reflect a defect in bone regulation instead of dietary intake. Further clinical and observational studies exploring the potential roles of calcium and phosphate in cancer should take into account their regulators such as vitamin D, parathyroid hormone, and fibroblast growth factor 23 (FGF-23) [164, 165] in order to fully comprehend how they are involved in carcinogenesis.

Table 5. Epidemiological studies on bone metabolism and cancer.

Publication	Study population	Study design	No. of subjects, follow-up	Exposure	Outcome	Main results	Adjustments
Brandstedt, 2012 [159]	The Malmo Diet and Cancer Study cohort, Sweden, men, born in 1923–1945	Case control	943 cases, 943 controls	Serum total calcium	Prostate cancer	OR: 1.34 (0.78–1.39) for highest versus lowest quartile	Matched on BMI, educational level, alcohol consumption, and smoking.
Schwartz, 2012 [160]	National Health and Nutrition Examination Survey III (NHANES III), USA, age 18+	Cohort	6707 at baseline, 49 events, 1069327 person-months	Serum total calcium	Prostate cancer mortality	HR: 1.50 (95% CI: 1.04–2.17) for every unit increase	Adjusted for age and BMI, serum albumin, and serum 25-OHD and account for survey weights and the complex sampling design of NHANES III
Salem, 2013 [161]	Iran, men, mean age 71.1 (cases) and 66.5 (controls)	Case control	194 cases, 317 controls	Serum total calcium	Prostate cancer	OR: 0.27 (0.12–0.59) for or highest versus lowest tertile	Adjusted for age, body mass index, occupation, educational level, smoking, alcohol, family history of prostate cancer, and sex hormones. Similar results with albumin-corrected calcium

Conclusion

Overall, more recent observational studies supported previous findings obtained in AMORIS, although no new results have been reported for serum fructosamine and inorganic phosphate with respect to cancer risk. A drawback of using serum markers in predicting risk of cancer is its potential fluctuations following other pathological conditions, resulting in non-specificity and imprecision of associations observed. Utilisation of multiple combination markers may provide benefit from enhanced specificity in relation to cancer, as well as repeated or serial measurements instead of a single measurement. Associations with other diseases may also necessitate further analytical strategies addressing effects of serum metabolic markers on competing events in addition to cancer. Finally, delineating the role of serum metabolic markers may generate valuable information to complement emerging clinical studies on preventive effects of drugs and supplements targeting metabolic disorders against cancer.

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Appendix A2. Prediagnostic serum inflammatory markers in relation to breast cancer risk, severity at diagnosis and survival in breast cancer patients. Published in Carcinogenesis, 2015.

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Prediagnostic serum inflammatory markers in relation to breast cancer risk, severity at diagnosis and survival in breast cancer patients

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Abstract

Inflammation has been linked to cancer but its role in breast cancer is unclear. We investigated common serum markers of inflammation: C-reactive protein (CRP), albumin, haptoglobin and white blood cells (WBC) in relation to breast cancer incidence, severity and survival. A total of 155 179 women aged 20 and older without any history of cancer were selected from a large Swedish cohort. Hazard ratios (HRs) for breast cancer were estimated with Cox regression, adjusting for potential confounders. Ordered and binomial logistic regression models were used to assess the associations of serum inflammatory markers with breast cancer severity and oestrogen receptor (ER) positivity at diagnosis, on the other. Cumulative incidence functions by levels of inflammatory markers were assessed for early death from breast cancer and all causes. During a mean follow-up of 18.3 years, 6606 women were diagnosed with breast cancer, of whom 1474 died. A positive association with incident breast cancer was seen for haptoglobin ≥ 1.4 g/l [HR 1.09; 95% confidence interval (CI): 1.00–1.18] compared to lower levels. No association was observed between inflammatory markers and breast cancer severity or ER positivity. Higher haptoglobin was linked to risk of early death from breast cancer (HR: 1.27, 95% CI: 1.02–1.59), whereas higher risk of early death from all causes was additionally found with CRP ≥ 10 mg/l (HR: 1.19, 95% CI: 1.04–1.36) and WBC ≥ 10×10^9/l (HR: 1.57, 1.14–2.16). Our findings indicate that prediagnostic serum inflammatory markers were weakly linked to incident breast cancer but corresponded to worse survival after diagnosis.

Introduction

Two distinct mechanisms link inflammation and cancer. Chronic inflammation may initiate or promote cancer through generation of reactive oxygen species and proinflammatory cytokines; and conversely, inflammation may occur secondary to cancer and affect disease progression (1). For breast cancer, the latter has been shown by the infiltration of white blood cell (WBC) components such as CD8+ and CD4+ regulatory T lymphocytes in cancer tissues, which has been linked to overall survival in breast cancer patients (2–4). The role of inflammation preceding breast cancer diagnosis and its prognostic implications are less clear.

Observational studies have increasingly explored the link between inflammation and incident breast cancer through the

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Abbreviations

AMORIS	Apolipoprotein Mortality Risk
BMI	body mass index
CRP	C-reactive protein
HRs	hazard ratios
WBC	white blood cells

use of systemic inflammatory markers. Most of these studies utilized C-reactive protein (CRP), an acute-phase reactant excessively produced by the liver during inflammation (5–9). In previous work, we assessed the risk of breast cancer in 59220 women and observed no statistically significant association between baseline serum levels of CRP or WBC and incident breast cancer (10). Our findings for CRP were later pooled in a meta-analysis with an additional 38330 women from five other prospective studies (11). This also yielded a null association, with a summary hazard ratio (HR) for breast cancer of 1.04 [95% confidence interval (CI): 0.91–1.17] with higher CRP levels, although measurement techniques and categorizations of CRP varied between studies. Additionally, long latency prior to clinical manifestation of breast cancer may affect the strength of the observed association (7).

More recently established markers of inflammation such as IL-6 and TNF-α have also been linked to cancer risk (12,13), but they are not routinely measured in clinic. Apart from CRP and WBC, other commonly used inflammatory markers are haptoglobin and albumin, the latter known to correlate inversely with inflammation (14). To further investigate the association between inflammation and incident breast cancer, we expanded our previous cohort in the Apolipoprotein Mortality Risk (AMORIS) Study by assessing haptoglobin and albumin in addition to CRP and WBC, including more participants (n = 155 179) and extended follow-up time. Furthermore, with the recent linkage to the Stockholm Clinical Quality Register for Breast Cancer, we were able to assess baseline levels of these markers in relation to breast cancer severity at time of diagnosis and survival in 6606 women who developed breast cancer.

Methods

Study population

The AMORIS study has been described in detail (15). Briefly, this cohort comprises Swedish men and women with blood samples sequentially sent to the Central Automation Laboratory (CALAB) in Stockholm, Sweden, during the period 1985–1996. Individuals recruited were mainly from the greater Stockholm area, who were either healthy and having a laboratory testing as part of a general check-up or outpatients referred for laboratory testing. None of the participants were inpatients at the time of sampling. In the AMORIS study, the CALAB database was linked to several Swedish national registries using the Swedish 10-digit personal identity number. Following a recent update, the AMORIS study now includes laboratory measurements of 812073 individuals with information on breast tumour characteristics from 1985 onwards (16) and follow-up information until 31 December 2011.

From the updated AMORIS database, we selected all women aged 20 and older with baseline serum CRP and albumin. All participants with any prior history of cancer were excluded. To exclude reverse causation, we only included women with follow-up time of more than 2 years. A total of 155179 women were therefore included in the final analysis (Supplementary Figure 1, available at *Carcinogenesis* Online). The study complied with the Declaration of Helsinki and was approved by the Ethics Review Board of the Karolinska Institute.

Definition of outcomes

Breast cancer diagnosis was obtained from the Swedish National Cancer Register and the Stockholm Clinical Quality Register of Breast Cancer, which has 97% coverage following validation against the records of the National Cancer Register. We used information on age at diagnosis, tumour stage and ER status to classify breast cancer cases into three levels of severity: mild, moderate and severe (Supplementary Table 1, available at *Carcinogenesis* Online). This classification was based on St. Gallen criteria (17) and corresponds to different survival profiles of all breast cancer patients in this study (Supplementary Figure 2, available at *Carcinogenesis* Online). Information on overall and breast cancer-specific mortality was obtained from the Swedish Cause of Death Register.

Assessment of exposures and covariates

Serum CRP and haptoglobin levels were measured with an immunoturbidimetric assay (reagents from Orion Diagnostics, Finland) using fully automated multichannel analyzers (for CRP an AutoChemist-PRISMA, New Clinicon, Sweden, 1985–1992 and a DAX 96, Technicon Instruments, USA, 1993–1996; for haptoglobin Hitachi-analyzers, Boehringer Mannheim, Germany) (15). High-sensitivity CRP was not available at any time in the period of blood sample collection (1985–1996), and CRP concentrations <10 mg/l could not be measured precisely (15). However, this cutoff of 10 mg/l was widely accepted as the upper limit of the health-associated reference range (18). Albumin was measured with a bromcresol green method and WBC count obtained by routinely used haematology analyzers (STKS Haematology System from Coulter Corporation, Hialeah, FL). Total imprecision calculated by the coefficient of variation was 12% at CRP level 40 mg/l, 5.6% at haptoglobin level 1.1 g/l, albumin <1.8 and <2.7% at WBC 10×10^9/l (19). Weight and height measurements were assessed in 24927 participants and body mass index (BMI) was calculated. Methods were fully automated and accredited (15).

Information on socioeconomic status (SES; white collar, blue collar, not gainfully employed or unknown) was collected from the Population and Housing Census for 1970–1990 (10). Information on parity (nulliparous, 1+) was obtained from the Swedish Multi-Generation Register, whereas information on hospitalization for liver disease, rheumatic disease, diabetes and lung disease (ever, never) was taken from the National Patient Register. From the Stockholm Clinical Quality Register for Breast Cancer, we obtained information on menopausal status at diagnosis (premenopausal, postmenopausal), tumour TNM stage based on the American Joint Committee on Cancer (AJCC) Cancer Staging Manual 7th edition (I, II, III, IV) and oestrogen receptor (ER) status (positive, negative). Period of cancer diagnosis was categorized (before 1989, 1989–1993, 1993–1997, 1997 onwards) to account for the long period of recruitment and differences in early cancer detection and management over time.

Statistical analysis

Levels of serum inflammatory markers were assessed as high or low based on their clinical cutoffs used in the CALAB laboratory: CRP 10 mg/l, haptoglobin 1.4 g/l and WBC 10×10^9/l. For albumin, a cutoff point of 40 g/l was used instead of 35 g/l due to the small number of participants with low albumin levels. Follow-up time was defined as the time from the baseline measurements until the date of breast cancer diagnosis, death from any cause, emigration or end of study, whichever occurred first. Hazard ratios and CIs for incident breast cancer were obtained with Cox proportional hazards regression, comparing women with high to low levels of CRP, albumin, haptoglobin and WBC. The assumption of proportional hazards was met by assigning variables as time-varying covariates. All models were adjusted for age at baseline measurements, SES and parity. Additional adjustments were performed for diabetes and lung disease as a proxy for smoking. A subanalysis based on menopausal status at baseline used age as a proxy of menopause. In the analysis of premenopausal women, individuals were followed to age 50 after which they were censored. In the assessment of postmenopausal risk, individuals with measurements before age 50 entered the study at age 50 by means of delayed entry (20). Since obesity is linked to inflammation and breast cancer (21,22), we also repeated our analysis in the subgroup of women with baseline BMI while adjusting for BMI. Additionally, since disease of the liver may impair production of CRP, albumin and haptoglobin (23,24), we performed a sensitivity analysis excluding 521 women with history of liver disease. A similar sensitivity analysis was performed by excluding 1436 women with history of rheumatic disease since levels of CRP and haptoglobin may be influenced by the disease and its medications (25).

To assess the association between prediagnostic inflammatory markers and breast cancer severity, ordered logistic regression was used to estimate proportional odds ratios of more severe breast cancer by categories of CRP, albumin, haptoglobin and WBC. This analysis allowed the use of three severity categories as ordered outcomes and was performed in 5108 breast cancer patients with available information on disease severity. The proportional odds assumption was met for all markers. The models were adjusted for age and menopausal status at diagnosis, period of diagnosis and interval time between baseline measurements and breast cancer diagnosis. Additionally, we used logistic regression to analyse inflammatory markers in relation to ER positivity as an outcome in the same subset of breast cancer patients.

Finally, we investigated prediagnostic inflammatory markers in relation to all-cause and breast cancer-specific death in 6606 women with breast cancer. Patients were followed up until death, emigration or end of study, whichever occurred first. Cox proportional hazards models were used, adjusting for age and menopausal status at diagnosis, tumour stage, ER status, period of diagnosis and interval time between baseline measurements and breast cancer diagnosis. Missing variables were assigned as a different value for menopausal status (18%), tumour stage (18%) and ER status (32%). To further illustrate this association, cumulative incidence functions for all-cause and breast cancer-specific death are displayed by categories of CRP, albumin, haptoglobin and WBC. Gray's test for equality of cumulative incidence functions was performed to assess differences in cumulative risk of death with respect to baseline markers.

All analyses were conducted with Statistical Analysis Systems (SAS) release 9.4 (SAS Institute, Cary, NC) and R version 3.0.2 (R Foundation for Statistical Computing).

Results

Baseline characteristics of study participants are shown in Table 1. During a mean follow-up of 18.3 years, 6606 women were diagnosed with breast cancer, of whom 1474 died, with breast cancer being the main cause of death in 736. Most of the study participants were gainfully employed. Higher parity was seen in those who developed breast cancer. Women with breast cancer also had higher levels of CRP and haptoglobin at baseline compared to those without.

Higher haptoglobin levels were associated with incident breast cancer (Table 2). This association slightly weakened in the fully adjusted model, showing a borderline increased risk (HR: 1.09, 95% CI: 1.00–1.18). No differences were observed when the model was further adjusted for diabetes and lung disease (results not shown). When the analysis was stratified based on age of 50 years as a proxy for menopause, a positive association was noted between CRP and breast cancer risk in premenopausal women (HR: 1.08, 95% CI: 1.08–1.30), whereas haptoglobin was associated with breast cancer risk only in postmenopausal women (HR: 1.09, 95% CI: 1.00–1.19). After adjustment for BMI in the subgroup of women with baseline BMI, the only association observed was between haptoglobin and incident breast cancer in postmenopausal women (HR: 1.24, 95% CI: 1.01–1.51 for high versus low levels of haptoglobin). Findings were similar when we excluded women with prior history of liver or rheumatic disease (results not shown).

Figure 1 shows the proportions of breast cancer severity with respect to categories of prediagnostic CRP, albumin, haptoglobin and WBC, where no marked difference was observed between women with high and low levels of these markers. A lack of association was also found between these markers and the odds of being diagnosed with more severe breast cancer (Table 3). Similarly, there were no differences observed in the odds of being diagnosed with ER-positive breast cancers with higher serum inflammatory markers (results not shown).

High prediagnostic haptoglobin was linked to increased risk of dying from breast cancer (HR: 1.27, 95% CI: 1.02–1.59). A similar association was seen with CRP, but disappeared after adjustment for other covariates. For all-cause mortality, a stronger association with prediagnostic markers was found (Table 4). Women with higher levels of CRP, haptoglobin and WBC are at greater risk of early death from any causes, with HR of 1.19 (95% CI: 1.04–1.36), 1.34 (1.15–1.55) and 1.57 (1.14–2.16) for high versus low levels of CRP, haptoglobin and WBC, respectively. An inverse association for albumin was not apparent after adjustment for other covariates.

Table 1. Baseline characteristics of study population by breast cancer status

	Breast cancer (*N* = 6606)	No breast cancer (*N* = 148573)
Age (years) – Mean (SD)	50.33 (11.56)	46.26 (14.78)
Mean follow-up (years) – Mean (SD)	11.72 (5.48)	18.58 (4.43)
Parity		
Nulliparous	1511 (22.87)	46781 (31.49)
1+	5095 (77.13)	101792 (68.51)
SES		
White collar	2930 (44.35)	54935 (36.98)
Blue collar	3178 (48.11)	74558 (50.18)
Unemployed or unknown	498 (7.54)	19080 (12.84)
History of liver disease	18 (0.27)	503 (0.34)
Body mass index (kg/m²)[a]		
<18.5	23 (2.05)	733 (3.08)
18.5–25	739 (65.86)	16002 (67.22)
25–30	262 (23.35)	5333 (22.40)
≥30	98 (8.73)	1737 (7.30)
CRP (mg/l)		
<10	5586 (84.56)	128354 (86.39)
≥10	1020 (15.44)	20219 (13.61)
Albumin (g/l)		
<40	880 (13.32)	17680 (11.90)
≥40	5726 (86.68)	130893 (88.10)
Haptoglobin (g/l)[b]		
<1.4	4118 (86.44)	84221 (87.60)
≥1.4	646 (13.56)	11918 (12.40)
WBC (10^9/l)[c]		
<10	2131 (94.08)	49762 (93.82)
≥10	134 (5.92)	3280 (6.18)

Measured in [a]24297, [b]100903 and [c]55307 women.

The association between prediagnostic serum inflammatory markers and death following breast cancer diagnosis was further demonstrated with cumulative incidence functions. A statistically significant higher cumulative risk of dying from breast cancer was observed in women with higher prediagnostic CRP and haptoglobin compared to those with lower levels (Figure 2). Similar but stronger trends were seen with all-cause death. Additionally, an inverse trend was observed between albumin and all-cause mortality over time.

Discussion

We found a borderline positive association between baseline haptoglobin and incident breast cancer. Although no association was observed between inflammatory markers and breast cancer severity at diagnosis or ER positivity, haptoglobin was positively linked to breast cancer death. Breast cancer patients with higher levels of CRP or haptoglobin or lower albumin levels prediagnostically were also shown to be more likely to die early from any causes.

Molecular pathways linking inflammation and breast cancer have been increasingly studied. Proinflammatory cytokines

Table 2. Hazard ratios and 95% confidence intervals for breast cancer risk by levels of serum inflammatory markers

	No. of breast cancer/ total participants	HR (95% CI)		No. of breast cancer/ total participants[b]	HR (95% CI)
		Crude	Adjusted[a]		Adjusted[b,c]
All women					
CRP (mg/l)					
<10	5586/133 940	1.00 (Ref)	1.00 (Ref)	976/21 972	1.00 (Ref)
≥10	1020/21 239	1.03 (0.97–1.10)	0.99 (0.92–1.06)	146/2955	0.95 (0.80–1.14)
WBC (10^9/l)					
<10	2131/51 893	1.00 (Ref)	1.00 (Ref)	179/3881	1.00 (Ref)
≥10	134/3414	0.99 (0.83–1.17)	1.07 (0.90–1.28)	12/254	1.10 (0.61–1.99)
Albumin (g/l)					
<40	880/18 560	1.00 (Ref)	1.00 (Ref)	170/3370	1.00 (Ref)
≥40	5726/136 619	0.88 (0.82–0.94)	0.97 (0.91–1.05)	952/21 557	0.92 (0.79–1.09)
Haptoglobin (g/l)					
<1.4	4118/88 339	1.00 (Ref)	1.00 (Ref)	798/17 587	1.00 (Ref)
≥1.4	646/12 564	1.19 (1.10–1.30)	1.09 (1.00–1.18)	124/2233	1.20 (0.99–1.45)
Premenopause[d]					
CRP (mg/l)					
<10	2882/84 511	1.00 (Ref)	1.00 (Ref)	559/14 588	1.00 (Ref)
≥10	497/11 663	1.22 (1.11–1.34)	1.18 (1.08–1.30)	79/1774	1.14 (0.90–1.44)
WBC (10^9/l)					
<10	891/28 729	1.00 (Ref)	1.00 (Ref)	95/2391	1.00 (Ref)
≥10	71/2073	1.14 (0.89–1.45)	1.04 (0.81–1.32)	6/164	0.84 (0.37–1.92)
Albumin (g/l)					
<40	370/9241	1.00 (Ref)	1.00 (Ref)	91/2058	1.00 (Ref)
≥40	3009/86 933	0.81 (0.72–0.90)	0.92 (0.83–1.02)	547/14 304	0.87 (0.70–1.09)
Haptoglobin (g/l)					
<1.4	2266/55 976	1.00 (Ref)	1.00 (Ref)	482/11 832	1.00 (Ref)
≥1.4	248/5956	1.18 (1.03–1.34)	0.94 (0.83–1.07)	56/1225	1.04 (0.79–1.38)
Postmenopause[d]					
CRP (mg/l)					
<10	4730/109 139	1.00 (Ref)	1.00 (Ref)	817/18 767	1.00 (Ref)
≥10	893/18 560	1.00 (0.93–1.07)	1.00 (0.93–1.07)	129/2633	0.99 (0.82–1.20)
WBC (10^9/l)					
<10	1845/42 027	1.00 (Ref)	1.00 (Ref)	155/3304	1.00 (Ref)
≥10	115/2810	1.03 (0.85–1.24)	1.06 (0.88–1.28)	9/231	0.95 (0.48–1.86)
Albumin (g/l)					
<40	792/16 325	1.00 (Ref)	1.00 (Ref)	145/2953	1.00 (Ref)
≥40	4831/111 374	0.93 (0.87–1.01)	0.95 (0.88–1.03)	801/18 447	0.93 (0.78–1.12)
Haptoglobin (g/l)					
<1.4	3524/75 302	1.00 (Ref)	1.00 (Ref)	666/15 190	1.00 (Ref)
≥1.4	589/11 617	1.09 (1.00–1.19)	1.09 (1.00–1.19)	114/2084	1.24 (1.01–1.51)

[a]Adjusted for age, SES (white collar, blue collar, unemployed or unknown) and parity (nulliparous, 1+).
[b]Subcohort analysis in women with baseline BMI.
[c]Adjusted for age, SES (white collar, blue collar, unemployed or unknown), parity (nulliparous, 1+) and BMI (<18.5, 18.5–25, 25–30 and ≥30 kg/m^2).
[d]Age of 50 years was used as a proxy for menopause. In the analysis of pre-menopausal women, individuals were followed to age 50 after which they were censored. In the assessment of post-menopausal risk, individuals with a baseline measurement taken before age 50 entered the study at age 50 by means of delayed entry. Note that this analysis allowed the same participants to be included in both groups, which resulted in a difference between the total numbers from pre-and post-menopausal analyses with the actual total numbers of women in the cohort.

released during inflammation may trigger the activation of signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappa B (NF-κB) signalling pathways, leading to activation of genes responsible for cell survival, proliferation and angiogenesis (26,27). Aberrant activations of STAT3 and NF-κB have been widely implicated in breast carcinogenesis (28,29) and jointly contribute to an immunosuppressive tumour microenvironment (30). Additionally, suppressor of cytokine signalling 3 (SOCS3), an inhibitor of cytokine production, negatively regulates STAT3 expression and decreases proliferation in breast cancer cells, further linking inflammation and breast cancer (31). Apart from its effects on carcinogenesis, STAT3 upregulates expression of acute-phase reactants including CRP (32,33) and haptoglobin (34), and a synergistic effect of NF-κB on this mechanism has been shown. These common regulatory pathways suggest that systemic markers of inflammation may be useful in studying the association between inflammation and breast carcinogenesis.

Our findings linking CRP and incident breast cancer showed a null association, which is in agreement with most previous studies (6,9–12,35–37). So far, a positive association between serum CRP and breast cancer risk has only been documented in three studies (5,7,8), where the largest number of breast cancer cases was 218. In addition to sample size, adjustments for potential confounding factors may explain the differences in estimates, especially BMI, since obesity has been suggested to

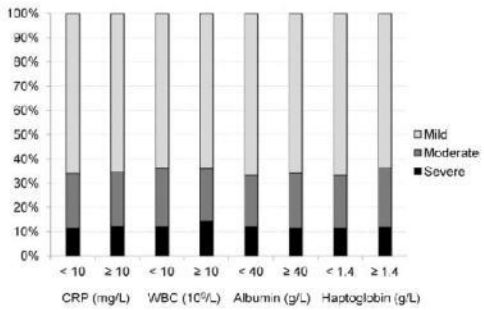

Figure 1. Proportion of breast cancer severity categories by levels of prediagnostic inflammatory markers.

Table 3. Proportional odds ratios and 95% confidence intervals for more severe breast cancer by levels of prediagnostic serum inflammatory markers

	Proportional OR (95% CI)	
	Crude	Adjusted[a]
CRP (mg/l)		
<10	1.00 (Ref)	1.00 (Ref)
≥10	1.04 (0.89–1.21)	1.04 (0.88–1.23)
WBC (10^9/l)		
<10	1.00 (Ref)	1.00 (Ref)
≥10	1.03 (0.70–1.52)	1.08 (0.73–1.60)
Albumin (g/l)		
<40	1.00 (Ref)	1.00 (Ref)
≥40	1.03 (0.87–1.21)	1.02 (0.86–1.22)
Haptoglobin (g/l)		
<1.4	1.00 (Ref)	1.00 (Ref)
≥1.4	1.13 (0.93–1.37)	1.14 (0.94–1.39)

[a]Adjusted for age and menopausal status at diagnosis (premenopause, postmenopause, unknown), period of diagnosis (1986–1995, 1995–1999, 1999–2003, 2003–2007, 2007–2011), interval time between measurement and breast cancer diagnosis.

Table 4. Hazard ratios and 95% confidence intervals for deaths in breast cancer patients by levels of prediagnostic serum inflammatory markers

	No. of death/total participants	HR (95% CI)	
		Crude	Adjusted[a]
Breast cancer-specific death			
CRP (mg/l)			
<10	597/5584	1.00 (Ref)	1.00 (Ref)
≥10	139/1022	1.22 (1.01–1.46)	1.16 (0.95–1.41)
Albumin (g/l)			
<40	113/878	1.00 (Ref)	1.00 (Ref)
≥40	623/5728	0.88 (0.72–1.08)	0.92 (0.75–1.13)
Haptoglobin (g/l)			
<1.4	417/4107	1.00 (Ref)	1.00 (Ref)
≥1.4	95/655	1.31 (1.05–1.64)	1.27 (1.02–1.59)
WBC (10^9/l)			
<10	240/2132	1.00 (Ref)	1.00 (Ref)
≥10	17/1332	1.23 (0.75–2.01)	1.23 (0.75–2.03)
All-cause death			
CRP (mg/l)			
<10	1162/5584	1.00 (Ref)	1.00 (Ref)
≥10	312/1022	1.38 (1.21–1.56)	1.19 (1.04–1.36)
Albumin (g/l)			
<40	256/878	1.00 (Ref)	1.00 (Ref)
≥40	1218/5728	0.78 (0.68–0.89)	0.95 (0.83–1.09)
Haptoglobin (g/l)			
<1.4	914/4107	1.00 (Ref)	1.00 (Ref)
≥1.4	219/655	1.55 (1.34–1.80)	1.34 (1.15–1.55)
WBC (10^9/l)			
<10	573/2132	1.00 (Ref)	1.00 (Ref)
≥10	42/132	1.25 (0.92–1.71)	1.57 (1.14–2.16)

[a]Adjusted for age and menopausal status at diagnosis (premenopause, postmenopause, unknown), TNM stage (I, II, III, IV, unknown), ER status (positive, negative, unknown), period of diagnosis (1986–1995, 1995–1999, 1999–2003, 2003–2007, 2007–2011), interval time between measurement and breast cancer diagnosis.

underlie the association between chronic inflammation and breast cancer (38). Nevertheless, only one study showed substantial effect modification by overweight status (BMI </≥ 25 kg/m^2) (37), whilst adjustment for BMI and other variables apart from age, such as hormonal factors, mostly had little impact on findings (6–9,12). Similarly, we found no marked difference in our overall results when adjusting for BMI in the subset of women with baseline BMI, but the association between CRP and incident breast cancer in premenopausal women was no longer seen. This may imply that obesity-related inflammation in younger age plays a more important role in breast carcinogenesis compared to when it occurs later in life. However, there is not enough evidence suggesting that this could be translated to inflammation in general as no differences were seen for other markers upon BMI adjustment.

Despite the borderline association observed with incident breast cancer, risk estimates from haptoglobin were more robust than from CRP when adjusted for BMI, suggesting that the marker is less affected by obesity. To our knowledge, this is the first study exploring the association between haptoglobin and breast cancer in the population setting. Previously, haptoglobin has mostly been investigated in the context of breast cancer treatment, where a decreased serum expression in response to endocrine therapy was observed (39–41). In contrast, Dowling *et al.* (42) compared the serum expression of haptoglobin in 33 breast cancer patients and 15 healthy females and found no statistically significant differences. The timing of measurements and the characteristics of participants, however, were not addressed. Given the paucity of evidence, our findings indicate a potential area of research with serum haptoglobin as a marker linking inflammation to incident and fatal breast cancer.

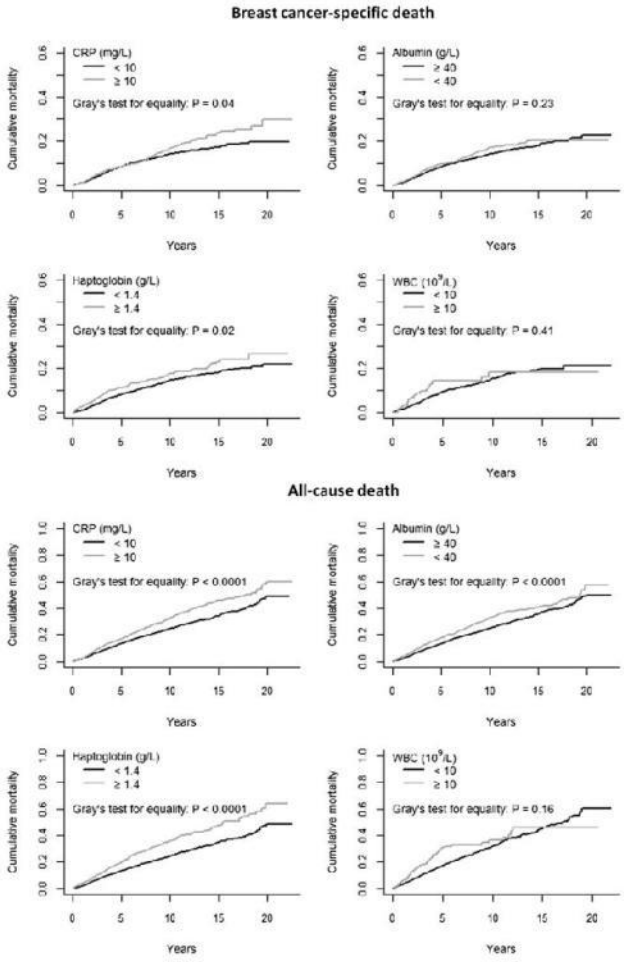

Figure 2. Cumulative mortality risk in breast cancer patients by levels of prediagnostic serum inflammatory markers. Note the different axes used in breast cancer-specific and all-cause death..

In our study, only prediagnostic haptoglobin showed a consistent association with breast cancer-specific death. Nevertheless, we found an increased risk of early death from all causes following breast cancer diagnosis in women with higher levels of CRP, haptoglobin and WBC, and a higher cumulative risk of all-cause mortality with lower albumin levels. This suggests better overall survival in breast cancer patients with normal levels of these markers prior to diagnosis. Allin and colleagues also studied prediagnostic CRP and all-cause mortality in 202 women diagnosed with breast cancer (36), and found no statistically significant association (HR: 1.1, 95% CI: 0.2–6.7 for CRP >3 mg/l compared to <1 mg/l, P_{trend} = 0.17). Besides the different measurement methods, this discrepancy may be accounted for by the low number of cases in the previous study, rendering the risk difference between prediagnostic CRP levels unquantifiable. In addition, there is evidence that postdiagnostic serum CRP may be associated with worse cancer-specific and overall survival in breast cancer patients (43–46), which indicates a prognostic value of serum inflammatory markers in addition to their role in breast cancer aetiology as indicated in the present study. Future mechanistic investigations and clinical studies are thus necessary to explore their implication in underlying mechanisms of breast cancer and management of the disease.

To date, this is the largest prospective study assessing common serum inflammatory markers in relation to breast cancer. All analyses were performed at the same laboratory, and complete follow-up was obtained for all participants. The population in the AMORIS study was selected by analysing fresh blood samples from health check-ups in non-hospitalized persons. However, any healthy worker effect would not influence the internal validity of our study. One of the limitations of our study

is that high-sensitivity CRP was not available at the time measurements were conducted. Therefore, we could not quantify any CRP values below 10 mg/l, which may have resulted in an underestimation of the association between serum CRP and breast cancer. We excluded women with measurements of serum inflammatory markers taken less than 2 years prior to breast cancer diagnosis to lessen the possibility of reverse causation. However, breast cancer may develop years before diagnosis, and this long latency may have had greater impact during earlier years of recruitment when screening tests were less common. Therefore, we adjusted our analysis for period of diagnosis to account for difference in early detection and management of breast cancer over time. There was no information on menopausal status or hormonal replacement therapy at baseline, but we accounted for hormonal factors by assessing parity and stratified our analysis using age as a proxy for menopause. In our study, higher parity was observed in women who had breast cancer, which opposes the well-accepted association between parity and breast cancer risk (3). Nevertheless, such discrepancy has been observed in several other Swedish cohorts (4,5), and may be driven by differences in other sociodemographic factors beyond the scope of the present study. Due to a high proportion of missing values for other tumour characteristics in the AMORIS study, only age at diagnosis, tumour stage and ER status were used to determine severity of breast cancer at diagnosis. Finally, systemic inflammation has been linked to other causes of death such as cardiovascular disease (47), which may have driven the strong association with all-cause death and underestimated the effects of inflammatory markers on breast cancer-specific death. Future studies should tailor methods to better assess how inflammation is specifically linked to breast cancer in the context of both risk prediction and prognosis.

Conclusion

In addition to being weakly linked to incident breast cancer, higher prediagnostic serum haptoglobin levels were associated with a higher risk of death from the disease, which supports a role of inflammation in breast carcinogenesis. Additionally, women with higher prediagnostic CRP, haptoglobin or WBC levels are at higher risk of dying early from any causes following breast cancer diagnosis. Our analyses imply that inflammation preceding breast cancer may impact survival after diagnosis. These findings suggest the importance of inflammation as one of the mechanisms underlying breast cancer which may be further investigated for possible intervention strategies in breast cancer patients.

Supplementary material

Supplementary Table 1 and Figures 1 and 2 can be found at http://carcin.oxfordjournals.org/

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Conflict of Interest Statement: N.H. is employed by the AstraZeneca Mölndal, Sweden and the views of the present study are his own and not necessarily any official views of the AstraZeneca. All remaining authors have declared no conflicts of interest.

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Appendix A3. Atopy and cancer risk: Insights from a prospective study on serum specific and total immunoglobulin E. Poster presented at the 2015 European Society for Medical Oncology (ESMO) Asia Congress, December 2015. Awarded with an ESMO Travel Grant.

Atopy and cancer risk: Insights from a prospective study on serum immunoglobulin E

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Background

- Atopy, or predisposition to immunoglobulin E (IgE)-mediated allergic reaction, reflects biased T helper 2 (T_H2) immune responses.
- Biased T_H2 responses have been implicated in cancer, but the association between atopy and cancer is unclear.
- Diverse measurement methods and retrospective or cross-sectional assessments of atopy may explain discrepancy in prior studies.
- We studied prospectively measured atopy against common inhalant allergens in relation to cancer risk and survival in a cohort with up to 25 years of follow-up.

Methods

- The Swedish Apolipoprotein Mortality Risk Study (AMORIS)
- A total of 8,727 cancer-free men and women aged 20+ with baseline measurements of specific IgE against inhalant allergens and total IgE
- Main exposures: atopy status (specific IgE ≥0.35 kU/L) and categories of specific IgE scores (0, 1-2, 3-4, 5-6) with test for trend
- Specific IgE as measured by Pharmacia CAP® system and total IgE by enzyme-linked immunosorbent assay (ELISA)

Statistical Analysis

- Multivariable Cox regression to examine prediagnostic specific IgE in relation overall cancer and ten most common cancer sites
- Chronological age as timescale
- Adjustment for socioeconomic status, history of chronic respiratory disease and asthma, year of measurement, and total IgE levels
- Kaplan-Meier curves to assess cancer survival

Results

Atopy and cancer incidence

- A total of 689 (7.9%) participants were diagnosed with cancer during a median follow-up of 18.6 years.
- Mean age at baseline was 40 years, the majority (69%) were females
- The most common three types of cancer were prostate, female breast, and colorectal cancer (**Table 1**).
- Levels of specific IgE corresponded to total IgE (**Figure 1**).

Table 1. Baseline characteristics of study participants by atopy status

	Atopy	
	No (*N* = 4,714)	Yes (*N* = 4,013)
Age (years)		
Mean (SD)	42.26 (13.74)	37.31 (12.66)
Sex, male, Nr (%)	1661 (35.24)	1945 (48.47)
Socioeconomic status, Nr (%)		
High	1912 (40.56)	1423 (35.46)
Low	2074 (44.00)	1697 (42.49)
Unclassified/missing	728 (15.44)	893 (22.25)
History of chronic respiratory disease, Nr (%)	67 (1.42)	92 (2.29)
Year of measurement, Nr (%)		
1992-1994	1192 (25.29)	986 (24.57)
1994-1996	2682 (56.89)	1997 (49.76)
1996	840 (17.82)	1030 (25.67)
Total IgE (kU/L)		
<25	1765 (37.44)	285 (7.10)
25-100	1937 (41.09)	1355 (33.77)
≥100	1012 (21.47)	2373 (59.13)
Mean follow-up in years, Mean (SD)	15.86 (3.59)	16.05 (3.23)
Any cancer during follow-up, Nr (%)		
All cancer	443 (9.40)	246 (6.13)
Breast (female)	115 (2.44)	50 (1.25)
Prostate	55 (1.17)	42 (1.05)
Colorectal	43 (0.91)	21 (0.52)
Gynaecological	41 (0.87)	12 (0.30)
Haematological	31 (0.66)	22 (0.55)
Melanoma	27 (0.57)	10 (0.25)
Pulmonary	21 (0.45)	14 (0.35)
Bladder	14 (0.30)	11 (0.27)
NMSC	16 (0.34)	9 (0.22)
CNS	11 (0.23)	9 (0.22)
Kidney	12 (0.25)	9 (0.22)

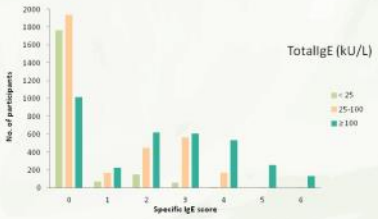

Figure 1. Distribution of specific and total IgE levels

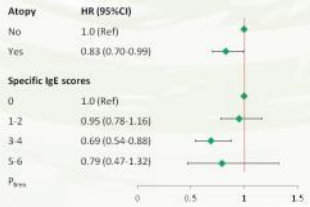

Figure 2. Associations between atopy, specific IgE levels, and risk of overall cancer

- An inverse association between atopy status and overall cancer incidence after adjustment for total serum IgE (**Figure 1**)
- A similarly inverse trend when using serum specific IgE categories (P_{trend} = 0.007)
- Stratification analyses: statistically significant associations only in women and in those with total IgE levels ≥100 kU/L
- Site-specific analyses showed an inverse association between serum specific IgE scores and risk of melanoma in men and women combined (P_{trend} = 0.04), and with female breast and gynaecological cancers combined (P_{trend} = 0.008).
- Sensitivity analyses adjusting for no. of allergens tested or no. of positive results → similar results

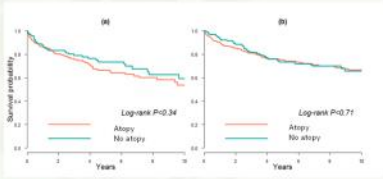

Figure 3. Kaplan-Meier assessing overall survival after cancer diagnosis by prediagnostic atopy status in men (a) and women (b)

Atopy and cancer survival

- Among all participants with cancer, 194 died with 146 persons had cancer as the primary cause of death.
- There was no difference in overall survival between prediagnostic atopy status (**Figure 3**) or serum specific IgE scores and death from all causes or from cancer.
- No association with cancer-specific death (results not shown)

Conclusion

- An inverse association between atopy or levels of specific IgE against inhalant allergens and risk of cancer, particularly in women and in those with high total IgE levels.
- Associations were driven by findings in melanoma and female breast and gynaecological cancer.
- No impact of prediagnostic atopy on survival after cancer diagnosis.

→ Potential role of T_H2-biased immune response in carcinogenesis, indicated by a shift in the balance between circulating IgE and other immunoglobulin classes.

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Appendix A4. Investigating the association between allergen-specific immunoglobulin E, cancer risk and survival. Published in Oncoimmunology, 2016.

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Investigating the association between allergen-specific immunoglobulin E, cancer risk and survival

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ABSTRACT

Prior findings linking allergy and cancer have been inconsistent, which may be driven by diverse assessment methods. We used serum specific immunoglobulin E (IgE) against common inhalant allergens that was assessed prior to cancer diagnosis in studying this association. We selected 8,727 Swedish men and women who had measurements of serum allergen-specific IgE and total IgE between 1992 and 1996. Multivariable Cox regression using age as a timescale was performed to assess the associations of IgE sensitization, defined by any levels of serum specific IgE ≥35 kU/L, with risk of overall and specific cancers. A test for trend was performed by assigning scores derived from allergen-specific IgE levels at baseline as an ordinal scale. Kaplan–Meier curves and log-rank test were used to assess cancer survival by IgE sensitization status. During a mean follow-up of 16 year, 689 persons were diagnosed with cancer. We found an inverse association between IgE sensitization and cancer risk, with a hazard ratio (HR) of 0.83 and 95% confidence intervals (CI) of 0.70–0.99. A similar trend was seen with specific IgE scores overall (P_{trend} = 0.007) and in women (P_{trend} = 0.01). Although IgE sensitization was not associated with risk of common site-specific cancers, serum specific IgE scores were inversely associated with melanoma risk in men and women combined, and with risk of female breast and gynecological cancers combined. No association with survival was observed. The association between circulating IgE levels and incident cancer may point toward a role of T-helper 2 (T_H2)-biased response in development of some cancers.

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Introduction

Allergy is a hypersensitivity reaction initiated by specific immunologic mechanisms.[1] Accruing evidence has indicated that allergy may be associated with the development of cancer, and is largely divided into two opposing views: (1) allergy may reduce cancer risk and (2) it may increase cancer risk.[2] The first may be explained by the immunosurveillance hypothesis, which states that increased immune surveillance following hyper-reactive immune responses may hinder the development of cancer.[2] Similarly, the prophylaxis hypothesis suggests that physical effects of allergy symptoms may prevent cancer by removal of potential carcinogens.[3] The opposing hypotheses include a shift in T-helper balance, which determines the type of immune responses elicited. Predominance of T_H2 over T_H1 underlies the hypersensitivity reactions in allergy, and is thought to divert immune responses from the tumor-eradicating T_H1 counterpart.[4] Additionally, allergic inflammation may lead to initiation and promotion of cancer directly or through indirect mechanisms.[5]

Observational findings linking allergy and cancer are inconclusive.[6] A crude way to determine presence of allergy would be using total IgE levels in the serum that may reflect the imbalance in the immune system, but without knowing its specificity makes the association between allergy and cancer challenging.[7] Atopy, which refers to the genetic pre-disposition of developing IgE-mediated hypersensitivity or IgE sensitization against allergens,[1] is often evaluated to unpick the role of allergy. Most previous studies crudely classified individuals into "atopic" and "non-atopic" based on self-reported history,[8-10] which relies on an individual's recall and may incur a loss of time-specific information. Other studies defined atopy based on laboratory evidence, such as the presence of circulating IgE.[1] The use of serum IgE against specific allergen provides more insight into how IgE sensitization may be associated to cancer, but may be hampered by the use of imprecise and/or different assessment methods. Findings based on pre-cancer diagnostic levels of

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Table 1. Characteristics of study participants by IgE sensitization status.

	IgE sensitization	
	No (n = 4,714)	Yes (n = 4,013)
Age (years)		
Mean (SD)	42.26 (13.74)	37.31 (12.66)
Sex, male, n (%)	1661 (35.24)	1945 (48.47)
Socioeconomic status, n (%)		
High	1912 (40.56)	1423 (35.46)
Low	2074 (44.00)	1697 (42.49)
Unclassified/missing	728 (15.44)	893 (22.25)
History of chronic respiratory disease, n (%)	67 (1.42)	92 (2.29)
Year of measurement, n (%)		
1992–1994	1192 (25.29)	986 (24.57)
1994–1996	2682 (56.89)	1997 (49.76)
1996	840 (17.82)	1030 (25.67)
Total IgE (kU/L)		
<25	1765 (37.44)	285 (7.10)
25–100	1937 (41.09)	1355 (33.77)
≥100	1012 (21.47)	2373 (59.13)
Mean follow-up in years, Mean (SD)	15.86 (3.59)	16.05 (3.23)
Any cancer during follow-up, n (%)		
All cancer	443 (9.40)	246 (6.13)
Breast (female)	115 (2.44)	50 (1.25)
Prostate	55 (1.17)	42 (1.05)
Colorectal	43 (0.91)	21 (0.52)
Gynecological	41 (0.87)	12 (0.30)
hematological	31 (0.66)	22 (0.55)
Melanoma	27 (0.57)	10 (0.25)
Pulmonary	21 (0.45)	14 (0.35)
Bladder	14 (0.30)	11 (0.27)
NMSC	16 (0.34)	9 (0.22)
CNS	11 (0.23)	9 (0.22)
Kidney	12 (0.25)	9 (0.22)

n = number of participants.

allergen-specific IgE are limited and no studies have investigated the impact on cancer survival of pre-diagnostic allergen-specific IgE.[11-13]

In the Swedish Apolipoprotein Mortality Risk Study (AMORIS), we previously investigated the association between total IgE and the risk of cancer in 24,820 individuals. A weak inverse association was found albeit not statistically significant, and results were similar when data from our cohort was combined in a meta-analysis with previous findings.[7] The recently updated AMORIS database now contains information on serum specific IgE utilizing comparable determinations, more participants, and a longer follow-up (up to 25 y). To gain further insight into the association between allergy and cancer, we now assessed serum specific IgE against common inhalant allergens in relation to risk of developing cancer and death after cancer diagnosis.

Results

Characteristics of study participants by IgE sensitization status are shown in Table 1. The average age at baseline was 40 y, and over half the study population were female (59%). During follow-up (median: 18.6 y), 689 incident cancer cases were identified. A total of 194 individuals died following cancer diagnosis, among which 146 died from cancer. The most common three types of cancer were prostate, female breast and colorectal cancers. The relative distribution of specific IgE scores to total IgE is shown in Table S1.

We assessed cancer risk based on IgE sensitization status and specific IgE scores as categories Table 2. When using

Table 2. Specific IgE scores in CALAB and corresponding serum concentrations.

Specific IgE score	Serum concentrations (kU/L)	Serum IgE levels
0	<0.35	Absent/undetectable
1	0.35 – 0.70	Low level
2	0.70 – 3.50	Moderate level
3	3.50 – 17.5	High level
4	17.5 – 50	Very high level
5	50 – 100	Very high level
6	≥100	Very high level

0.35 kU/L as the cut-off point for specific IgE, which indicates IgE sensitization, we did not observe any association with risk of cancer (Table 3). However, a statistically significant inverse trend was observed when using serum specific IgE scores in the overall study population ($P_{trend} = 0.03$). In sex stratification, a similar association was observed in women. Adjustment for serum total IgE showed stronger associations and an inverse association between IgE sensitization and cancer risk in the overall population (HR: 0.83 (95% CI: 0.70–0.99). No association was noted in men and women separately. When stratifying the analyses by serum total IgE levels, the inverse trend was only seen between serum allergen-specific IgE scores and cancer risk in the overall population and in women with total IgE levels >100 kU/L (Table 3). Similar associations were observed when additionally adjusting our model for the number of allergen tested, the number of positive results, or the ratio between the two (results not shown).

Similar associations to overall cancers were found when assessing all cancers excluding NMSC (Table 4). No statistically significant association was found between positive IgE sensitization and risk of specific cancer types. When observing trends across allergen-specific IgE scores, we found a lower risk of melanoma with higher specific IgE in both men and women combined ($P_{trend} = 0.04$). No association was observed for other cancer sites. To further investigate the driver of the inverse association observed in women, we combined cancers of female genital organs (breast and gynecological cancers) as a single outcome and a protective effect of higher specific IgE scores was observed ($P_{trend} = 0.04$).

In our secondary analysis, we assessed risk of death following cancer diagnosis. As shown by the Kaplan–Meier curves in Fig. 1, the probability of survival was lower in men with IgE sensitization compared to those without in the long-term follow-up, but there were no statistically significant difference (Log-rank $p > 0.05$). Similarly, no differences were observed when categories of allergen-specific IgE scores were used (results not shown). We further evaluated this association by conducting Cox regression and found no clear associations between IgE sensitization or specific IgE scores and death from all-causes or cancer, e.g. HR for cancer death was 1.04 (95% CI: 0.59–1.84) and 1.54 (0.91–2.62) for men and women with compared to without IgE sensitization, respectively (results not shown in tables).

Discussion

In the present study, IgE sensitization was associated with a lower risk of incident cancer. The inverse trend between allergen-specific IgE was more pronounced in women and among

Table 3. Associations between IgE sensitization, serum specific IgE scores and risk of incident cancer overall and by sex with chronological age as timescale.

		HR (95% CI)						
		IgE sensitization		Specific IgE scores				
	n cancer/n total	No	Yes	0	1–2	3–4	5–6	P_{trend}
Both men and women								
n		4714	4013	4714	6402	8331	8727	
Multivariable model	689/8727	1.0 (Ref)	0.88 (0.75–1.03)	1.0 (Ref)	1.00 (0.82–1.21)	0.74 (0.59–0.93)	0.91 (0.55–1.51)	0.03
Additional adjustment for total IgE	689/8727	1.0 (Ref)	0.83 (0.70–0.99)	1.0 (Ref)	0.95 (0.78–1.16)	0.69 (0.54–0.88)	0.79 (0.47–1.32)	0.007
Stratification by total IgE (kU/L)								
	191/2050	1.0 (Ref)	0.79 (0.48–1.32)	1.0 (Ref)	0.86 (0.51–1.47)	0.48 (0.12–1.99)	N/A	0.29
25–100	238/3292	1.0 (Ref)	0.85 (0.64–1.13)	1.0 (Ref)	0.91 (0.65–1.28)	0.77 (0.51–1.16)	N/A	0.18
≥100	260/3385	1.0 (Ref)	0.82 (0.63–1.06)	1.0 (Ref)	1.00 (0.74–1.34)	0.66 (0.48–0.91)	0.81 (0.47–1.39)	0.02
Men								
n		1661	1945	1661	732	1003	210	
Multivariable model	277/3606	1.0 (Ref)	0.97 (0.76–1.23)	1.0 (Ref)	1.01 (0.75–1.36)	0.89 (0.65–122)	1.27 (0.64–2.52)	0.80
Additional adjustment for total IgE	277/3606	1.0 (Ref)	0.89 (0.69–1.17)	1.0 (Ref)	0.95 (0.69–1.29)	0.83 (0.59–1.16)	1.10 (0.54–2.23)	0.42
Stratification by total IgE (kU/L)								
<25	53/649	1.0 (Ref)	0.71 (0.29–1.76)	1.0 (Ref)	0.59 (0.20–1.78)	1.11 (0.27–4.67)	N/A	0.64
25–100	97/1377	1.0 (Ref)	0.83 (0.54–1.28)	1.0 (Ref)	0.99 (0.59–1.66)	0.68 (0.38–1.23)	N/A	0.24
≥100	127/1580	1.0 (Ref)	0.97 (0.67–1.41)	1.0 (Ref)	1.00 (0.64–1.57)	0.89 (0.57–1.40)	1.23 (0.58–2.61)	0.94
Women								
n		3053	2068	3053	956	926	186	
Multivariable model	412/5121	1.0 (Ref)	0.83 (0.67–1.03)	1.0 (Ref)	0.98 (0.76–1.27)	0.64 (0.45–0.90)	0.72 (0.34–1.53)	0.01
Additional adjustment for total IgE	412/5121	1.0 (Ref)	0.81 (0.64–1.02)	1.0 (Ref)	0.96 (0.74–1.24)	0.61 (0.42–0.87)	0.64 (0.30–1.39)	0.01
Stratification by total IgE (kU/L)								
<25	138/1401	1.0 (Ref)	0.82 (0.44–1.53)	1.0 (Ref)	0.98 (0.53–1.83)	N/A	N/A	0.30
25–100	141/1915	1.0 (Ref)	0.87 (0.59–1.26)	1.0 (Ref)	0.85 (0.54–1.34)	0.91 (0.52–1.58)	N/A	0.51
≥100	133/1805	1.0 (Ref)	0.73 (0.52–1.04)	1.0 (Ref)	0.98 (0.66–1.46)	0.51 (0.31–0.83)	0.63 (0.29–1.39)	0.01

[§]Highest specific IgE scores recorded at baseline.
N/A = not applicable; n = number of participants.
All models were adjusted for sex (except for sex-specific analysis), socioeconomic status, period of measurement and history of chronic pulmonary disease.

Table 4. Associations between IgE sensitization, serum specific IgE scores and risk of site-specific cancers by sex with chronological age as timescale.

		HR (95% CI)						
		IgE sensitization		Specific IgE scores[§]				
	n cancer	No	Yes	0	1–2	3–4	5–6	P_{trend}
Both men and women								
All excluding NMSC	664	1.0 (Ref)	0.82 (0.69–0.99)	1.0 (Ref)	0.96 (0.78–1.18)	0.67 (0.52–0.85)	0.81 (0.48–1.36)	0.005
Colorectal	64	1.0 (Ref)	0.71 (0.39–1.26)	1.0 (Ref)	0.75 (0.38–1.51)	0.60 (0.27–1.34)	1.23 (0.28–5.42)	0.32
hematological	53	1.0 (Ref)	0.95 (0.51–1.76)	1.0 (Ref)	1.16 (0.58–2.30)	0.66 (0.27–1.59)	1.22 (0.27–5.46)	0.62
Melanoma	37	1.0 (Ref)	0.53 (0.24–1.17)	1.0 (Ref)	0.79 (0.33–1.88)	0.32 (0.09–1.13)	N/A	0.04
Pulmonary	35	1.0 (Ref)	0.87 (0.41–1.84)	1.0 (Ref)	1.24 (0.56–2.76)	0.41 (0.11–1.43)	0.94 (0.12–7.44)	0.33
Bladder	25	1.0 (Ref)	1.20 (0.50–2.87)	1.0 (Ref)	1.45 (0.56–3.77)	0.95 (0.29–3.14)	N/A	0.89
NMSC	25	1.0 (Ref)	0.97 (0.40–2.39)	1.0 (Ref)	0.62 (0.17–2.20)	1.56 (0.55–4.44)	N/A	0.74
Kidney	21	1.0 (Ref)	1.09 (0.40–2.95)	1.0 (Ref)	1.50 (0.52–4.30)	0.27 (0.03–2.23)	3.42 (0.63–18.50)	0.95
CNS	20	1.0 (Ref)	1.33 (0.47–3.73)	1.0 (Ref)	1.82 (0.62–5.33)	0.88 (0.21–3.61)	N/A	0.74
Men								
All excluding NMSC	267	1.0 (Ref)	0.89 (0.68–1.16)	1.0 (Ref)	0.95 (0.69–1.31)	0.80 (0.57–1.13)	1.12 (0.55–2.28)	0.37
Prostate	97	1.0 (Ref)	0.93 (0.38–1.04)	1.0 (Ref)	0.86 (0.50–1.48)	0.93 (0.53–1.63)	2.31 (0.78–6.82)	0.79
Colorectal	35	1.0 (Ref)	0.71 (0.34–1.49)	1.0 (Ref)	0.77 (0.31–1.88)	0.54 (0.19–1.53)	1.83 (0.38–8.77)	0.52
hematological	23	1.0 (Ref)	1.13 (0.44–2.89)	1.0 (Ref)	1.11 (0.36–3.35)	1.12 (0.35–3.62)	1.35 (0.15–12.15)	0.78
Pulmonary	15	1.0 (Ref)	1.07 (0.35–3.26)	1.0 (Ref)	1.41 (0.42–4.71)	0.57 (0.11–3.02)	2.38 (0.24–23.99)	0.93
Melanoma	12	1.0 (Ref)	0.56 (0.15–2.06)	1.0 (Ref)	0.65 (0.13–3.18)	5.31 (0.10–2.77)	N/A	0.31
Women								
All excluding NMSC	397	1.0 (Ref)	0.81 (0.63–1.03)	1.0 (Ref)	0.98 (0.75–1.28)	0.57 (0.39–0.83)	0.65 (0.30–1.42)	0.008
Breast and gynecological	218	1.0 (Ref)	0.76 (0.55–1.06)	1.0 (Ref)	0.85 (0.58–1.24)	0.68 (0.43–1.10)	0.36 (0.09–1.47)	0.04
Breast	165	1.0 (Ref)	0.83 (0.57–1.20)	1.0 (Ref)	0.95 (0.63–1.45)	0.69 (0.40–1.19)	0.49 (0.12–2.05)	0.14
Gynecological	53	1.0 (Ref)	0.55 (0.27–1.13)	1.0 (Ref)	0.54 (0.22–1.32)	0.65 (0.25–1.64)	N/A	0.11
Colorectal	29	1.0 (Ref)	0.75 (0.29–1.91)	1.0 (Ref)	0.75 (0.25–2.26)	0.84 (0.23–3.10)	N/A	0.53
Hematological	30	1.0 (Ref)	0.83 (0.37–1.88)	1.0 (Ref)	1.13 (0.48–2.71)	0.37 (0.08–1.65)	1.10 (0.14–8.82)	0.40
Melanoma	25	1.0 (Ref)	5.13 (0.19–1.42)	1.0 (Ref)	0.86 (0.30–2.43)	0.18 (0.02–1.49)	N/A	0.07

[§]Highest specific IgE scores recorded at baseline.
N/A = not applicable; n = number of participants; NMSC = nonmelanoma skin cancer; CNS = central nervous system.
All models were adjusted for sex (except for sex-specific analysis), socioeconomic status, period of measurement, history of chronic pulmonary disease and serum total IgE.

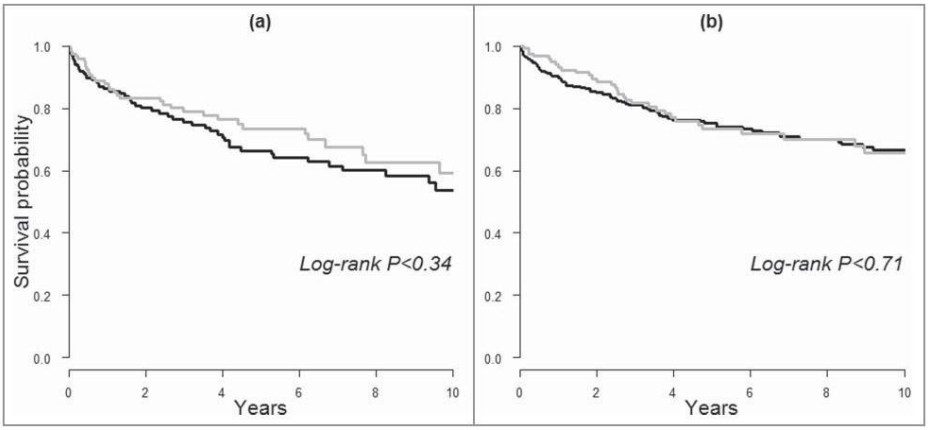

Figure 1. Kaplan–Meier curves of 10-y survival following cancer diagnosis in (A) men and (B) women based on prediagnostic IgE sensitization. Black lines indicate IgE sensitization and the gray lines indicate a lack thereof.

those with high total IgE levels. Among the most common cancers, no association was observed except for an inverse trend between serum specific IgE scores and risk of melanoma in the overall population, and with risk of breast and gynecological cancer in women. No associations between prediagnostic allergen-specific IgE and survival following cancer diagnosis were observed.

A shift toward immunosuppressive immune responses is characteristic of cancer.[22] Nevertheless, little is known about the role of humoral immune responses, particularly IgE, in carcinogenesis. IgE production and class-switch recombination (CSR) to IgE from other immunoglobulin types such as IgG are regulated by T_H2, and it has been suggested that a biased T_H2 response underlies high IgE levels in allergic individuals.[23] Since only limited responses following allergen exposure are observed for IgE,[24] it is possible that any impact on carcinogenesis is secondary to the biased T_H2 response rather than a result of high circulating IgE itself. In support of this, a temporal model of IgE and IgG has been proposed, in which the early-response IgE undergo sequential CSR to higher-affinity IgG3, then to IgG1, IgG2, and finally IgG4,[25-27] the latter of which displays low immunoactivatory functions. Furthermore, inflammatory T_H2-biased conditions such as IL-10, IL-4, VEGF and $FoxP3^+$ Tregs that support class switching to IgG4 rather than IgE, and elevated IgG4 levels have been reported in different tumors including melanoma.[28,29] Specifically in melanoma, elevated serum IgG4 levels and $IgG4^+$ B cells in patient circulation are associated with worse clinical outcomes, implying a bias toward an alternative rather than an IgE-biased response associated with melanoma cancer growth.[30,31] Taken together, these indications point toward a role of CSR dysregulation associated with T_H2-biased response in driving the link between allergy, circulating IgE, and IgG4-related diseases including some cancers.[32] Nevertheless, our findings in individuals with high total IgE ($\geq$100 kU/L) may be consistent with a potential requirement for a critical threshold toward a classical IgE, rather than an alternative IgG4-biased T_H2 immune response activation to confer for any potential protective benefits from cancer.

To date, only few prospective studies have examined possible associations between prediagnostic IgE sensitization and risk of incident cancer. In a recent study, Skaaby and colleagues evaluated serum specific evaluated IgE against inhalant allergen among 14,849 individuals.[11] A lack of association between allergen-specific IgE levels and risk of overall cancer was reported for IgE sensitization.[11] Similarly, we found a lack of a statistically significant association when assessing IgE sensitization against inhalant allergens in relation to overall incident cancer. However, when we took into account serum total IgE, an inverse association was found. Differences in follow-up periods and cohort composition may explain the discrepancy in the findings. Our study was based on a large cohort with a median follow-up of 18.6 y. In comparison, the study by Skaaby and colleagues comprised five cohorts spanning over different time periods, with a shorter overall median follow-up of 11.8 y.[11] Adjustments for other risk factors such as smoking, alcohol consumption and physical activity did not alter findings in that study and are thus unlikely to explain the discrepancy with the results in our study in which risk factor information was unavailable.

For specific cancers, observational findings seem to vary by demographics and timing of specific IgE measurements. Two European nested case-control studies demonstrated an inverse association between IgE sensitization against inhalant allergens and risk of glioma in women but not men,[13,33] whereas a lack of association was reported by another nested case-control study based on four US cohorts.[34] Besides population attributes, a smaller number of cases in the latter study may explain this inconsistency. In case-control studies where allergen-specific IgE in cases was assessed after diagnosis, IgE sensitization was inversely associated with risk of lymphoid malignancies and positively with prostate cancer risk.[35,36] However, no such association was observed in nested case-

control studies where serum samples were prospectively collected before diagnosis.[11,12,35]

In our study population, we found no associations between IgE sensitization and risk of specific cancer sites, which is comparable with the study conducted by Skaaby and colleagues.[11] However, an inverse association between serum specific IgE scores and risk of breast and gynecological cancers were observed in women. To date, evidence from observational studies on the role of allergy in these cancers remains unclear. In a meta-analysis, a lack of associations between history of any allergy, asthma or hay fever and breast cancer risk was suggested.[9] On the other hand, a reduced incidence of ovarian, endometrial and cervical cancers have been reported in allergic patients.[37-40] There is little evidence based on IgE sensitization status except for cancer of the uterus, where a lack of association was suggested.[11] Considering the influence of estrogen in the development of these cancers, these results may indicate an interplay between immunologic and hormonal factors in carcinogenesis. Interestingly, endocrine treatment agents for estrogen-positive (ER+) breast cancers such as tamoxifen has been shown to reduce allergen-specific immunoglobulin levels including IgE in animal models of atopic dermatitis,[41] which further suggests such complex associations.

There are several caveats in assessing serum allergen-specific IgE as a marker of allergy. Allergen-specific IgE levels represent the probability of having clinical allergic disease, therefore, use of a single allergen and/or cut-off to define IgE sensitization may not fully be representative of one's allergic symptoms.[42] In line with this notion, we found stronger associations with categories of specific IgE compared to the conventional single cut-off point of specific IgE levels, which indicates that specific IgE scores or categories may be more useful than a single cut-off point in assessing cancer risk. Additionally, it was suggested that the number of positive inhalant allergens correlates better with allergic diseases compared to a single positive allergen-specific IgE test.[43] In our study, we have addressed this possibility by an additional adjustment for the number of allergen tested, the number of positive results, or the ratio between the two, and they did not alter our findings. Finally, there is an indication that the sum of specific IgE levels against common inhalant allergens correlates better with clinical symptoms such as wheezing [44] and hospitalization with asthma,[45] compared to individual levels of specific IgE. We were unable to assess cumulative levels of specific IgE in this study. Therefore, future studies assessing allergy-related cancer susceptibility may benefit from refined criteria of IgE sensitization.

To date, this is the first study documenting the association between IgE sensitization to common inhalant allergens and the risk of cancer using both serum allergen-specific and total IgE, and also the first study investigating the impact of pre-diagnostic IgE on cancer survival. Strengths of our study include the prospectively collected allergen-specific and total IgE levels prior to the diagnosis of cancer. Complete follow-up was obtained and all laboratory measurements were performed in one and the same laboratory.[14] By using age as a timescale, we addressed the strong influence of age on absolute levels of specific IgE and its relative proportion to total IgE.[19,46] A limitation of this study is the lack of information on clinical symptoms of allergy. Although information on specific types of allergens was available, we were unable to link individual allergens with risk of cancer due to the lack of number of cases. Our study population only included individuals who underwent IgE testing as part of a check-up or as outpatients and therefore may not be representative of the general population. However, this is not expected to influence the internal validity of this study. Allergy symptoms may have been confused with smoking-related respiratory disorders. To account for the lack of information on smoking, we adjusted our analysis for history of hospitalization with chronic obstructive pulmonary disease and asthma. Nevertheless, residual confounding may still have occurred. Lastly, spurious correlations may be of concern when performing multiple comparisons as shown in our study. However, we planned our analyses based on prior evidence and our results are explicable by suggested biological pathways and findings from other studies. Therefore, the observed association is unlikely to be spurious, although a discrepancy with the strength of the true association is possible due to the lack of cases.

In summary, our study suggests that IgE sensitization is weakly associated with a lower risk of malignancy in cancer-free individuals. These findings add to the evidence that immune responses involved in allergy contribute to the susceptibility of being diagnosed with cancer, particularly female breast and gynecological cancers and melanoma. In particular, our results may support a role of T_H2-biased immune response in development of these cancers, indicated by a shift in the balance between circulating IgE and IgG subclasses including the low immunoactivatory IgG4, which urges further mechanistic investigations.

Methods

Study population

The AMORIS study has been described in detail elsewhere.[14] Briefly, this study includes Swedish men and women with blood samples sequentially sent to the Central Automation Laboratory (CALAB) in Stockholm, Sweden[14] Participants were either healthy and had a laboratory testing as a part of general health check-up, or were outpatients referred for laboratory testing. None of the participants were inpatients when samples were collected. In the AMORIS study, the CALAB database is linked to Swedish national registries, providing complete follow-up information on cancer diagnosis, death and emigration.[15]

Following a recent update, the AMORIS study now includes laboratory measurements of 812,073 individuals with follow-up information until 31 December 2011. From this population, we included 8,727 men and women aged 20 and older with no history of cancer who had baseline measurements of allergen-specific IgE against inhalant allergen and total IgE concentrations between 1992 and 1996. The study complied with the Declaration of Helsinki and was approved by the Ethics Review Board of the Karolinska Institutet.

Assessment of outcome

Cancer diagnosis was obtained from the population-based Swedish Cancer Register. International Classification of Diseases, seventh revision (ICD-7) codes were used to classify cancer sites. In addition to overall cancer (ICD-7: 140–207), we assessed all cancer

excluding non-melanoma skin cancer (NMSC) (ICD-7: 140–207 excluding 191) and the 10 most frequently diagnosed cancers in our study population: prostate (ICD-7: 177), female breast (ICD-7: 170), colorectal (ICD-7: 153–154), gynecological including ovarian, uterus and cervix (ICD-7: 171–176), hematological (ICD-7: 200–207), melanoma skin (ICD-7: 190), pulmonary (primary; ICD-7: 162), bladder (ICD-7: 181), NMSC (ICD-7: 191), central nervous system (CNS; ICD-7: 193) and kidney cancer (ICD-7: 180). The secondary outcomes of this study were all-cause and cancer-specific deaths. Dates and causes of death were obtained from the Swedish Cause of Death Register, whereas information on emigration was retrieved from the Migration Register.

Assessment of exposures and covariates

Specific IgE concentrations against common inhalant allergens were measured using immunoassay. The test system, Pharmacia CAP® System (Thermo Fisher Scientific, formerly Pharmacia Diagnostics AB, Uppsala, Sweden), is based on solid phase coupled allergen, adsorbing the IgE antibodies in the sample and assessed by an anti-IgE antibody commercially developed. It has been well standardized, shows correct quantitative values and is reproducible over time.[16] A list of inhalant allergens tested is available in Table S1. Results of allergen-specific IgE test were expressed as scores ranging from 0 to 6 which represent different levels of IgE from undetectable up to high concentrations of IgE (kU/L) as displayed in Table 2. Apart from these scores, no information on continuous levels of specific IgE was available. As with previous studies, any scores higher than 0 (which correspond to specific IgE levels of ≥0.35 kU/L) were defined as IgE sensitization and the presence of atopy.[17] For consistency, the term IgE sensitization was used to describe specific IgE levels ≥0.35 kU/L throughout this study. When multiple allergens were tested at baseline, results for all allergen-specific IgE measurements were collected and positive IgE sensitization was defined as having at least one positive result among all the tested allergens. In addition to IgE sensitization status, highest specific IgE scores recorded at baseline examinations when multiple allergens were tested were used in the analysis. Serum total IgE (kU/L) was measured by enzyme-linked immunosorbent assay (ELISA) using Immunoassay System ES 700 (Boehringer-Mannheim, Germany). Coefficient of variation was less than 5%. Total IgE levels were categorized based on the clinical cut-off points into low (<25 kU/L), moderate (25–100 kU/L) or high levels (≥100 kU/L) as previously described (173).

Age at baseline measurement (years) was collected from the CALAB database. The period of measurement was categorized (1992–1993, 1994–1995, 1996) to account for a long recruitment period. Socioeconomic status (SES; white collar, blue collar, unemployed or unknown) was based on national Censuses.[18] From the National Patient Register, we used data on history of hospitalization with chronic pulmonary disease including asthma (ever, never).

Statistical analysis

Multivariable Cox regression was used to estimate HR with corresponding 95% CI of overall risk of cancer by IgE sensitization status (yes, no) in all participants. Follow-up time was defined as the time from baseline measurement until cancer diagnosis, death from any cause, emigration or end of study, whichever occurred first. Additionally, the trend between allergen-specific IgE scores against inhalant allergens and risk of cancer was evaluated by assessing scores in groups (0, 1–2, 3–4, 5–6) as an ordinal scale. Levels of allergen-specific IgE are known to substantially decrease with age.[19] Therefore, all analyses were performed using age as the time scale with delayed entry.

Models were adjusted for sex, SES, and period of measurement, history of chronic pulmonary disease to account for asthma and as a proxy for smoking given their association to IgE sensitivity and risk of lung cancer.[20,21] Analyses were repeated in men and women separately. A further adjustment for categories of total IgE levels was performed in the second model. To evaluate any effect modification by total IgE levels, analyses were stratified according to total IgE levels. In an additional analysis, we adjusted our model for the number of allergen tested, the number of positive results or the ratio between the two. Analysis was subsequently performed for all cancer excluding NMSC and the 10 most common cancer sites with adjustment for total IgE levels. We repeated a similar analysis in men and women but only assessed the five sex-specific most common cancer sites due to the lack of number of cases.

For our secondary objective, we studied pre-diagnostic allergen-specific IgE in relation to survival after cancer diagnosis. Three cancer patients were excluded in the analysis because the diagnosis of cancer occurred at the time of death, leaving 686 individuals with cancer in the final analysis. Follow-up time was defined as the time from cancer diagnosis until death from any cause, emigration or end of study, whichever occurred first. Kaplan–Meier curves were used to assess overall survival by IgE sensitization status and scores of allergen-specific IgE, and statistical differences were assessed with the log-rank test. Cox regression was used to quantify the risks of all-cause and cancer-specific deaths by IgE sensitization status and allergen-specific IgE scores with age at diagnosis as time scale. The models were adjusted for the interval between baseline IgE measurements and cancer diagnosis, and total IgE levels.

All analyses were conducted with Statistical Analysis Software (SAS) release 9.4 (SAS Institute, Cary, NC) and R version 3.0.2 (R Foundation for Statistical Computing).

Disclosure of potential conflicts of interest

The authors declare that they have no competing interests. Niklas Hammar is employed by the AstraZeneca, but the views expressed in the manuscript are his own and not those of AstraZeneca.

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Appendix A5. Serum lactate dehydrogenase and survival following cancer diagnosis.

Poster presented at the 2015 European Cancer Congress, September 2015.

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Serum lactate dehydrogenase and survival following cancer diagnosis

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Background

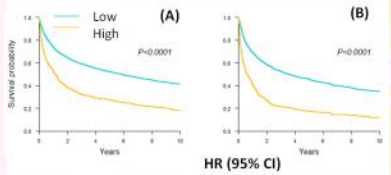
- Higher serum lactate dehydrogenase (LDH) may indicate poor prognosis, but most studies focused on overall instead of cancer survival.
- Current problems: non-specificity to cancer, high publication bias, lack of data from population studies with long-term follow-up.
- We studied the associations of serum LDH with all-cause and cancer-specific death in a cohort with up to 25 years of follow-up.

Methods

- The Swedish Apolipoprotein Mortality Risk Study (AMORIS)
- A total of 7,895 men and women aged 20+ with diagnosis of cancer during 1986-1999
- Baseline serum LDH assessed within 3 years before diagnosis
- LDH measured by spectrophotometry, high/low based on upper limit of normal (ULN)

Statistical Analysis

- Multivariable Cox regression to examine prediagnostic LDH levels in relation to risk of all-cause and cancer-specific death
- Analysis for specific cancer sites
- Stratification by lag time to diagnosis



	HR (95% CI)	
All death	1.66 (1.53 – 1.81)	1.91 (1.65 – 2.20)
Cancer death	1.72 (1.56 – 1.90)	2.06 (1.76 – 2.41)

Figure 1. Kaplan-Meier curves and Cox regression assessing survival by LDH levels measured (A) 3 years before diagnosis and (B) 3 months before or at diagnosis.

Results

- A total of 5,799 (73.5%) patients died during a mean follow-up of 8.2 years.
- Among all deaths, 4,222 had cancer as the primary cause of death.
- Mean age at diagnosis was 2 years, the majority (51.5%) were males.
- Participants with high levels of baseline LDH (>ULN) were older, had higher comorbidity burden, and lower 5-year overall survival rates.
- The most common cancers were breast (females), prostate, and colorectal cancers.

Cancer site	All-cause death: No. of deaths	All-cause death: HR (95% CI)	Cancer-specific death: No. of deaths	Cancer-specific death: HR (95% CI)
Breast*	579	1.76 (1.21–2.57)	301	1.61 (0.90–2.88)
Prostate†	713	2.19 (1.63–2.95)	403	2.43 (1.65–3.57)
Pulmonary	647	1.74 (1.34–2.25)	543	1.67 (1.25–2.22)
Colorectal	681	1.88 (1.47–2.40)	450	2.17 (1.64–2.89)
Gastroesophageal	304	2.08 (1.33–3.26)	258	1.99 (1.24–3.18)
Hepatobiliary	165	0.76 (0.51–1.13)	138	0.80 (0.52–1.23)
Pancreas	254	0.94 (0.64–1.36)	236	0.96 (0.65–1.42)
Kidney	183	1.11 (0.67–1.83)	122	1.33 (0.74–2.41)
Bladder	233	1.18 (0.64–2.16)	75	0.65 (0.20–2.17)
Gynaecological*	323	1.42 (0.97–2.09)	209	1.82 (1.19–2.80)
Head and neck	112	0.72 (0.32–1.60)	56	0.84 (0.28–2.49)
Melanoma	104	2.40 (1.16–5.00)	37	2.06 (0.58–7.29)
Central nervous system	211	1.64 (0.91–2.94)	139	1.28 (0.58–2.81)
Haematological	547	1.36 (1.09–1.69)	385	1.47 (1.14–1.89)

*Subanalysis in women; not adjusted for sex
†Subanalysis in men; not adjusted for sex

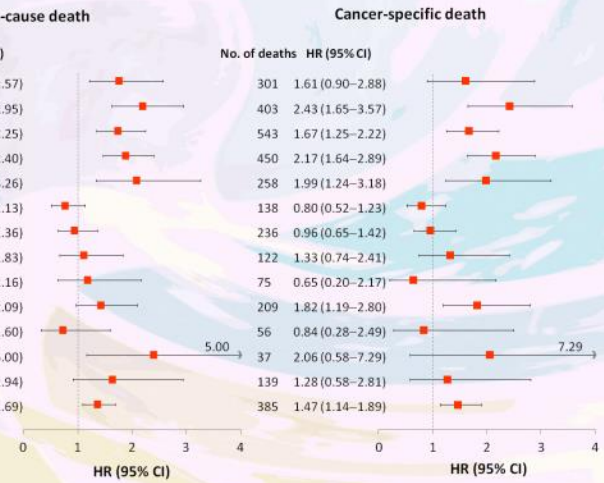


Figure 2. Hazard ratios and 95% confidence intervals for death following cancer diagnosis for high (>ULN) compared to low serum LDH (≤ULN) as the reference, stratified by cancer site. All Cox models were adjusted for age at diagnosis, sex, socioeconomic status, Charlson comorbidity index, and period of diagnosis.

- High serum LDH levels measured 3 years and 3 months prediagnostically corresponded to worse overall and cancer-specific survival (**Figure 1**).
- Associations were stronger when assessing cancer-specific death, and when LDH was measured within 3 months prior to diagnosis (N = 1,657).
- Site-specific analyses showed a positive association between serum LDH and cancer-specific death in patients diagnosed with prostate, pulmonary, colorectal, gastroesophageal, gynaecological and haematological cancers (**Figure 2**).

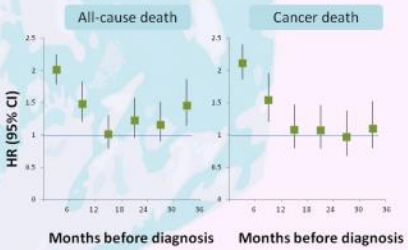


Figure 3. Association between LDH and survival by lag time between measurements and diagnosis (months).

- Stronger associations with survival was observed when serum LDH measurements were closer to cancer diagnosis (**Figure 3**).
- A positive association between LDH assessed 30-36 months before diagnosis and overall death (HR: 1.46, 95% CI: 1.15–1.86).
- Findings were similar in a sub-analysis for breast cancer adjusting for tumour stage.

Conclusion

- Our findings corroborate the association between serum LDH and worse survival in several types of cancer.
- Increasing LDH levels and stronger associations closer to diagnosis → relevance with tumour growth or progression.
- Differential associations between all-cause and cancer-specific death may represent relationships with other fatal diseases e.g. cardiovascular disease → potential use of LDH subunits or combination with tumour characteristics to improve its prognostic value.

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Appendix A6. Serum lactate dehydrogenase and survival following cancer diagnosis. Published in the British Journal of Cancer, 2015.

FULL PAPER

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Keywords: LDH; the Warburg effect; survival; prospective study

Serum lactate dehydrogenase and survival following cancer diagnosis

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Background: There is evidence that high level of serum lactate dehydrogenase (LDH) is associated with poorer overall survival in several malignancies, but its link to cancer-specific survival is unclear.

Methods: A total of 7895 individuals diagnosed with cancer between 1986 and 1999 were selected for this study. Multivariable Cox proportional hazards regression was used to assess overall and cancer-specific death by the z-score and clinical categories of serum LDH prospectively collected within 3 years before diagnosis. Site-specific analysis was performed for major cancers. Analysis was repeated by different lag times between LDH measurements and diagnosis.

Results: At the end of follow-up, 5799 participants were deceased. Hazard ratios (HRs) and 95% confidence intervals (CIs) for overall and cancer-specific death in the multivariable model were 1.43 (1.31–1.56) and 1.46 (1.32–1.61), respectively, for high compared with low prediagnostic LDH. Site-specific analysis showed high LDH to correlate with an increased risk of death from prostate, pulmonary, colorectal, gastro-oesophageal, gynaecological and haematological cancers. Serum LDH assessed within intervals closer to diagnosis was more strongly associated with overall and cancer-specific death.

Conclusions: Our findings demonstrated an inverse association of baseline serum LDH with cancer-specific survival, corroborating its role in cancer progression.

Aberrant energy metabolism is a common feature of cancer (Hanahan and Weinberg, 2011). In normal cells, when oxygen is available, pyruvate generated during the breakdown of glucose is utilised to produce energy through oxidative phosphorylation. In contrast, tumour cells prefer pyruvate metabolism via anaerobic pathway regardless of oxygen availability, leading to inefficient fuel production and formation of lactate. This anomalous metabolic preference is known as the Warburg effect or 'aerobic glycolysis' (Vander Heiden *et al*, 2009; Thorne and Campbell, 2014).

Lactate dehydrogenase (LDH) is the enzyme responsible for the conversion of pyruvate to lactate during glycolysis (Hirschhaeuser *et al*, 2011). It is expressed in all tissues and its A and B subunits, coded by two different genes *LDH-A* and *LDH-B*, combine to construct five isoenzymes (LDH1 to LDH5) with selective distribution among tissues and in serum (Maekawa, 1988). In addition, LDH is known as a marker for tissue injury, inflammation, haemolysis and myocardial infarction (Drent *et al*, 1996; Kemp *et al*, 2004; Kato *et al*, 2006). Elevated LDH levels are

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seen in cancer patients, and its prognostic value has been shown in several malignancies such as germ cell tumours, lymphoma, melanoma and renal cell carcinoma (Balch *et al*, 2004; Barlow *et al*, 2010; Armstrong *et al*, 2012; Nagle *et al*, 2013). Most of these studies were based in hospital or clinical trial settings, with LDH assessed at diagnosis or after initiation of treatment. However, recent evidence linking *LDH-A* and the oncogene *c-MYC* suggests that metabolic derangements may occur before the tumour becomes macroscopic, that is, before it is clinically detectable (Ferreira *et al*, 2012). The role of these early metabolic disturbances in the context of cancer survival remains poorly understood.

Currently, there is also a lack of information on how serum LDH contributes to cancer-specific survival in long-term follow-up (Petrelli *et al*, 2015). In a prospective cohort with up to 25 years of follow-up, we sought to investigate the association of prediagnostic serum LDH with overall and cancer-specific deaths among 7895 individuals diagnosed with cancer. We assessed serum LDH measured within 3 years before cancer diagnosis, during which the tumour is likely to be preclinically present (Shen and Zelen, 2001), and within 3 months before diagnosis, which represented its levels at the time of diagnosis. Furthermore, we investigated temporal associations between LDH and survival using LDH measured in different interval periods before cancer diagnosis.

MATERIALS AND METHODS

The Apolipoprotein MOrtality RISk (AMORIS) study has been described in detail elsewhere (Holme *et al*, 2010). Briefly, this study included Swedish men and women with blood samples sequentially sent to the Central Automation Laboratory (CALAB) in Stockholm, Sweden. This population was representative of the general Stockholm population (Holme *et al*, 2010). Participants were either healthy and had a laboratory testing as a part of general health check up, or were outpatients referred for laboratory testing. None of the participants were in-patients when samples were collected. In the AMORIS study, the CALAB database was linked to Swedish national registries, providing complete follow-up information. Following a recent update, the AMORIS study now includes laboratory measurements of 812 073 individuals with follow-up information until 31 December 2011. From this population, we selected 7895 men and women aged $\geqslant 20$ years with histopathological diagnosis of incident cancer between 1986 and 1999 who had prediagnostic serum LDH measurements. Follow-up time was defined as the time from cancer diagnosis until the date of death from any cause, emigration or end of study (31 December 2011), whichever occurred first. The study complied with the Declaration of Helsinki and was approved by the Ethics Review Board of the Karolinska Institutet.

Diagnosis of cancer and outcomes. Cancer diagnosis was obtained from the Swedish National Cancer Register and International Classification of Diseases, 7th revision (ICD-7) codes were used to classify major cancer sites. The outcomes of this study were overall death and cancer-specific death. The latter was based on information from the Swedish Cause of Death Register, using ICD-8 since the beginning of study until 30 December 1986, ICD-9 from 1 January 1987 to 31 December 1996 and ICD-10 codes afterwards (Supplementary Table S1). For specific cancer sites, cancer-specific deaths were defined as individuals whose primary cause of death matched their primary cancer diagnosis.

Assessment of exposure and covariates. Serum concentrations of LDH (μkat l^{-1}) were measured with an enzymatic spectrophotometric method (Holme *et al*, 2010) on automated multichannel analyzers (an AutoChemist-PRISMA, New Clinicon, Stockholm, Sweden, 1985–1992; and a DAX 96, Technicon Instruments Corporation, Tarrytown, NY, USA, 1993–1996) (Holme *et al*, 2010). Total imprecision calculated by the coefficient of variation was $<4\%$ for both analyzers. The method was fully automated with automatic calibration and accredited laboratory facilities (Holme *et al*, 2010). Prediagnostic LDH was defined as the last measurement taken within 3 years before cancer diagnosis. For a secondary analysis, we collected LDH measured within six 6-month intervals before cancer diagnosis and an average was calculated for individuals with >1 measurement within any interval time. We calculated the standardised value (z-score) of LDH by subtracting with the mean and dividing by the s.d. Both nontransformed LDH and its z-score were normally distributed. As LDH cutoffs vary across laboratories, we used its upper limit of normal (ULN) to categorise LDH into low and high levels ($\leqslant$ ULN and $>$ULN).

Socioeconomic status (white collar, blue collar, unemployed or unknown) was based on the national censuses (Wulaningsih *et al*, 2013a). We calculated Charlson comorbidity index (CCI) using information from the National Patient Register. The CCI consists of 17 groups of diseases with a specific weight assigned to each disease category (Wulaningsih *et al*, 2013a). These weights were then summed to obtain an overall score, resulting in four comorbidity levels (0, 1, 2 and 3+) indicating no comorbidity to severe comorbidity. Period of diagnosis was categorised (before 1989, 1989–1993, 1993–1997 and 1997 onwards) to account for the long period of recruitment and differences in cancer management over time. Information on tumour stage was available for 877 breast cancer cases from the Stockholm Breast Cancer Quality Register and was classified based on the American Joint Committee on Cancer (AJCC) Cancer Staging Manual 7th edition (Stages I to IV).

Statistical analysis. We used Kaplan–Meier curves to assess overall survival by categories of prediagnostic LDH, and statistical differences were assessed with the log-rank test. Cox proportional hazard regression was used to estimate hazard ratios (HRs) and their 95% confidence intervals (CIs) of overall and cause-specific death by z-score and categories of LDH, adjusting for age at diagnosis. In the multivariable model, we further adjusted for sex, socioeconomic status, CCI and period of diagnosis. We also performed a site-stratified model by assigning each major cancer site as an individual stratum and the remaining cancers as one additional stratum. To assess serum LDH at the time of diagnosis, we repeated our analyses in a subgroup of 1657 participants who had their baseline LDH measured within 3 months before cancer diagnosis.

To observe the association between baseline LDH and survival in specific cancers, we performed similar multivariable analysis by major cancer sites. For breast cancer there was information available on tumour stage, and hence that we repeated this analysis while adjusting for tumour stage. Cumulative incidence functions were used to display cumulative risk of dying from all-cause and cancer, and statistical difference was assessed with Gray's test for equality of cumulative incidence functions. We displayed Kaplan–Meier curves and cumulative incidences only for deaths up to 10 years after diagnosis as trends past this cutoff point were similar to the ones presented. However, statistical analyses were performed using data for the whole follow-up.

In a secondary analysis, we aimed to observe any temporal association between LDH and survival in cancer patients. Pearson's correlation coefficients (r) between LDH levels in different intervals were calculated. The average of LDH was measured for each 6-month time interval before cancer diagnosis and associations of LDH with overall and cancer-specific cancer survival for each lag time were examined. The models were adjusted for age at diagnosis, sex, socioeconomic status, CCI, period of diagnosis and stratified by cancer sites. A subset analysis was performed for breast cancer, stratified by tumour stage (I–II and III–IV).

All analyses were conducted with Statistical Analysis Software (SAS) release 9.4 (SAS Institute, Cary, NC, USA) and R version 3.0.2 (R Project for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics of study participants by LDH categories are shown in Table 1. Mean age at diagnosis was 62 years. At the end of follow-up (mean: 8.2 years), 5799 participants (73.5%) were deceased. Participants with high levels of baseline LDH (>ULN) were older and had higher comorbidity burden and lower 5-year overall survival rates. Distributions of serum LDH *z*-score based on characteristics of participants are available in (Supplementary Figure S1). The three most frequent cancers were breast (female), prostate and colorectal cancer.

Overall survival differed by LDH measured within 3 years before diagnosis and within 3 months before date of diagnosis, that is, LDH at the time at diagnosis, with lower survival seen with higher LDH (Figure 1). Correspondingly, multivariable Cox proportional hazards regression showed an increased risk of dying from all causes with higher LDH *z*-score or categories, with the HR of 1.78 (95% CI: 1.64–1.94) comparing LDH levels above and below ULN. Similar findings were found when assessing cancer-specific death, for example, the HR for overall cancer death was 1.85 (95% CI: 1.68–2.03) for high *vs* low LDH. Associations were slightly attenuated when the models were stratified by cancer site (Table 2). Similar but more evident associations were found in the subanalysis only including serum LDH at the time at diagnosis.

Table 1. Baseline characteristics of study participants by categories of serum LDH measured within 3 years before cancer diagnosis

	LDH			
	⩽ULN, *N* (%)		>ULN, *N* (%)	
	No.	%	No.	%
Age at diagnosis, years				
Mean (s.d.)	62.3 (12.7)		65.6 (12.9)	
Sex, %				
Male	3682	51.0	381	56.1
Female	3534	49.0	298	43.9
Socioeconomic status, %				
White collar	3136	43.5	237	34.9
Blue collar	2774	38.4	147	36.4
Not gainfully employed or unknown	1306	18.1	195	28.7
Charlson comorbidity index, %				
0	5871	81.4	525	77.3
1	871	12.1	82	12.1
2	252	3.5	37	5.5
3+	222	3.1	35	5.2
Period of diagnosis, %				
Before 1989	1480	20.5	166	24.5
1989–1993	2215	30.7	235	34.6
1993–1997	2399	33.3	229	33.7
1997 onwards	1122	15.6	49	7.2
Cancer site, %				
Breast	1081	15	37	5.5
Prostate	832	11.5	49	7.2
Pulmonary	598	8.3	70	10.3
Colorectal	784	10.9	82	12.1
Gastro-oesophageal	296	4.1	22	3.2
Hepatobiliary	130	1.8	36	5.3
Pancreas	222	3.1	35	5.2
Kidney	199	2.8	25	3.7
Bladder	335	4.6	14	2.1
Gynaecological	486	6.7	42	6.2
Head and neck	134	1.9	11	1.6
Melanoma	259	3.6	13	1.9
Central nervous system	302	4.2	14	2.1
Haematological	567	7.9	122	18
Median survival time, months	67.8		13.7	
5-Year overall survival, %	95.3		4.7	

Abbreviations: LDH = lactate dehydrogenase; ULN = upper limit of normal.

When specific cancer sites were assessed, higher risk of overall death was observed with high LDH in individuals diagnosed with breast, prostate, pulmonary, colorectal, gastro-oesophageal, melanoma and haematological cancer and melanoma (Figure 2). The strongest association was seen for prostate cancer (HR: 2.19, 95% CI: 1.63–2.95). Similar but weaker trends were found when assessing cancer-specific death, with a positive association between LDH and risk of dying from prostate, pulmonary, colorectal, gastro-oesophageal and haematological cancer. In addition, a positive association was seen with gynaecological cancer death. Results were similar when *z*-score was used (results not shown). In a subgroup analysis of 877 women with breast cancer and available information on tumour stage, adjustment for tumour stage did not alter the associations between LDH and death, with HR of all-cause and breast cancer death of 1.73 (95% CI: 1.12–2.67) and 1.54 (95% CI: 0.81–2.92), respectively, for high compared with low LDH levels (results not shown).

We further visualised the association between LDH and cancer with cumulative incidence functions (Figure 3) and found higher cumulative risks of dying from overall, prostate, pulmonary, colorectal, gastro-oesophageal, kidney, gynaecological and haematological cancer with high LDH levels. Interestingly, an inverse association was observed for head and neck cancer that approached statistical significance (Gray's test $P = 0.05$).

In our secondary analysis, LDH levels measured within 6-month time intervals before cancer diagnosis were found to be increasing in interval times closer to diagnosis for overall and several types of cancer such as hepatobiliary and haematological cancer (Supplementary Figure S2). The LDH measurements taken within different interval times were highly correlated ($r > 0.5$ and $P < 0.0001$; Supplementary Table S2). When risk of overall death in all participants was assessed for every interval time, a stronger association was observed with mean LDH measured closer to diagnosis, that is, within 1 year before diagnosis. However, we found an increased risk of early death in those with high LDH measured 30 to 36 months before diagnosis (HR: 1.46, 95% CI: 1.15–1.86). For cancer-specific death, associations were also stronger when LDH was measured closer to diagnosis (Table 3). Similarly, in patients with stage I to II breast cancer, we found a positive association between LDH measured 30 to 36 months before diagnosis and overall death (HR: 2.97, 95% CI: 1.38–6.39), and between LDH measured 6 to 12 months before diagnosis and breast cancer death (HR: 1.95, 95% CI: 1.24–16.00). Results in advanced stage of disease were hampered by a low number of events (results not shown).

DISCUSSION

In the present study, we found higher prediagnostic LDH to correspond to lower overall and cancer-specific survival following cancer diagnosis. More specifically, a greater risk of dying from cancer was seen with increasing LDH in those diagnosed with prostate, pulmonary, colorectal, gastro-oesophageal, gynaecological or haematological cancer. Furthermore, we found that the associations between LDH and both all-cause and overall cancer deaths were stronger when LDH was measured closer to cancer diagnosis.

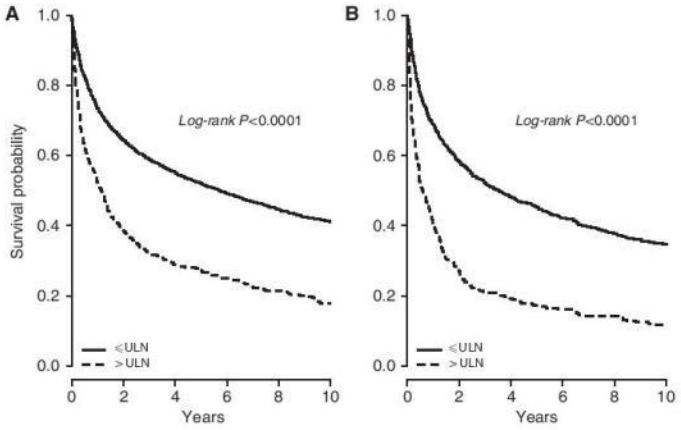

Figure 1. Kaplan–Meier curves for 10-year overall survival following cancer diagnosis by serum LDH levels measured (**A**) within 3 years before diagnosis and (**B**) within 3 months before diagnosis.

Table 2. Hazard ratios and 95% confidence intervals for associations between prediagnostic serum LDH and risk of death following cancer diagnosis

		LDH	
	Z-score	≤ULN	>ULN
All-cause death			
No. of deaths/all patients		5187/7216	612/679
Age adjusted	1.18 (1.16–1.21)	1.0 (Ref)	1.78 (1.64–1.94)
Multivariable[a]	1.16 (1.14–1.19)	1.0 (Ref)	1.66 (1.53–1.81)
Stratified by cancer site[a]	1.12 (1.10–1.15)	1.0 (Ref)	1.43 (1.31–1.56)
Sampling ⩽3 months before diagnosis[b]	1.15 (1.12–1.18)	1.0 (Ref)	1.91 (1.65–2.20)
Cancer-specific death			
No. of deaths/all patients		3760/7216	462/679
Age adjusted	1.19 (1.17–1.22)	1.0 (Ref)	1.85 (1.68–2.03)
Multivariable[a]	1.17 (1.14–1.20)	1.0 (Ref)	1.72 (1.56–1.90)
Stratified by cancer site[a]	1.12 (1.10–1.15)	1.0 (Ref)	1.46 (1.32–1.61)
Sampling ⩽3 months before diagnosis[b]	1.17 (1.12–1.20)	1.0 (Ref)	2.06 (1.76–2.41)

Abbreviations: LDH = lactate dehydrogenase; Ref = reference; ULN = upper limit of normal.
[a]Adjusted for age at diagnosis, sex, socioeconomic status, Charlson comorbidity index and period of diagnosis.
[b]Subanalysis in 1657 participants. Adjusted for age at diagnosis, sex, socioeconomic status, Charlson comorbidity index, period of diagnosis and stratified by cancer site.

Several plausible mechanisms may underlie the link between LDH and cancer progression. Rapidly proliferating cancer cells requires extreme supplies of energy and chronic hypoxia secondary to tumour growth activates hypoxia-inducible factor 1 (HIF-1), a key regulator of glycolysis and angiogenesis (Palmer and Clegg, 2014). The HIF-1 drives the metabolic switch to glycolysis by stimulating expression of glycolytic enzymes (Seagroves *et al*, 2001) and directly repressing mitochondrial function through activation of pyruvate dehydrogenase kinase 1 (PDK-1) (Kim *et al*, 2006; Papandreou *et al*, 2006). The subsequent accumulation of glycolytic metabolites may promote further HIF-1 activation, resulting in a feed-forward stimulatory loop in cancer cells (McFate *et al*, 2008). Hypoxia-inducible factor 1 also upregulates angiogenic factors including vascular endothelial growth factor-A (VEGF-A) (Pouysségur *et al*, 2006), therefore linking glycolysis and LDH to angiogenesis and cancer progression (Ostergaard *et al*, 2013; Parks *et al*, 2013). However, continuous oxygen availability in glycolytic cancers, such as leukaemia, suggests that other underlying factors may trigger the switch to aerobic glycolysis before hypoxia occurs (Vander Heiden *et al*, 2009). In addition, the tumour-promoting role of A and B subunits of LDH has been suggested: increased LDH-A levels are crucial in c-MYC-mediated cell transformation (Shim *et al*, 1997; Lewis *et al*, 2000), whereas LDH-B is necessary in mammalian target of rapamycin (mTOR)-mediated tumourigenesis (Zha *et al*, 2011). These biological findings imply that LDH may be relevant to tumour growth and severity, and may also play a role in carcinogenesis.

A prognostic value of serum LDH has been suggested in several types of cancer, particularly haematological malignancies. Serum LDH is a predictor of worse survival in diffuse large B-cell lymphoma (DBCL) and is one of the five risk factors included in the International Prognostic Index (IPI) (Nagle *et al*, 2013; Zhou *et al*, 2014). Similar associations with survival have been established in chronic myeloid and lymphocytic leukaemias (Weinberg *et al*, 2007; Goldaniga *et al*, 2008) and small cell lung cancer (SCLC) (You *et al*, 2008; Danner *et al*, 2010). In recent clinical trials, elevated serum LDH has been shown as an independent predictor of overall survival in advanced or metastatic cancer of the breast (Brown *et al*, 2012), prostate (Scher *et al*, 2009; Gravis *et al*, 2014), colorectum (Bar *et al*, 2014), oesophagus (Polee *et al*, 2003), pancreas (Tas *et al*, 2001), ovary (Schneider *et al*, 1998), nasopharynx (Jin *et al*, 2013), gastric adenocarcinoma (Sougioultzis *et al*, 2011), hepatocellular carcinoma (HCC) (Faloppi *et al*, 2014), renal cell carcinoma (Armstrong *et al*, 2012) and melanoma (Balch *et al*, 2004; Weide *et al*, 2012). The inverse association with overall survival in solid tumours were shown in a recent meta-analysis, with HR for overall death of 1.7 (95% CI: 1.62–1.79) (Petrelli *et al*, 2015). Nevertheless, a marked publication bias was observed, with most of smaller studies reporting only positive associations. Our study also found higher risks of early death among cancer patients with high levels of baseline serum LDH at the time of diagnosis and within 3 years before diagnosis. However, when we performed site-specific analysis with cancer-specific death as the outcome of interest, this association was only shown in those diagnosed with prostate, pulmonary, colorectal, gastro-oesophageal, gynaecological or haematological cancer. Considering the link of LDH with other chronic diseases that may contribute to death (Kemp *et al*, 2004; Kato *et al*, 2006), it is therefore important to consider cause-specific death to gain further insight into the prognostic relevance of LDH.

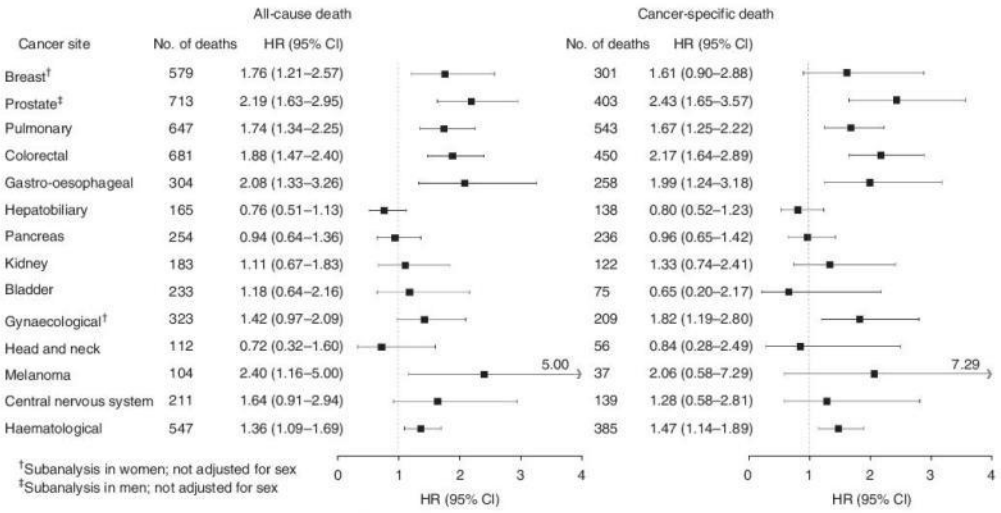

Figure 2. Hazard ratios and 95% confidence intervals for death following cancer diagnosis for high (>ULN) compared with low serum LDH (⩽ULN) as the reference, stratified by cancer site. All models were adjusted for age at diagnosis, sex, socioeconomic status, Charlson comorbidity index and period of diagnosis.

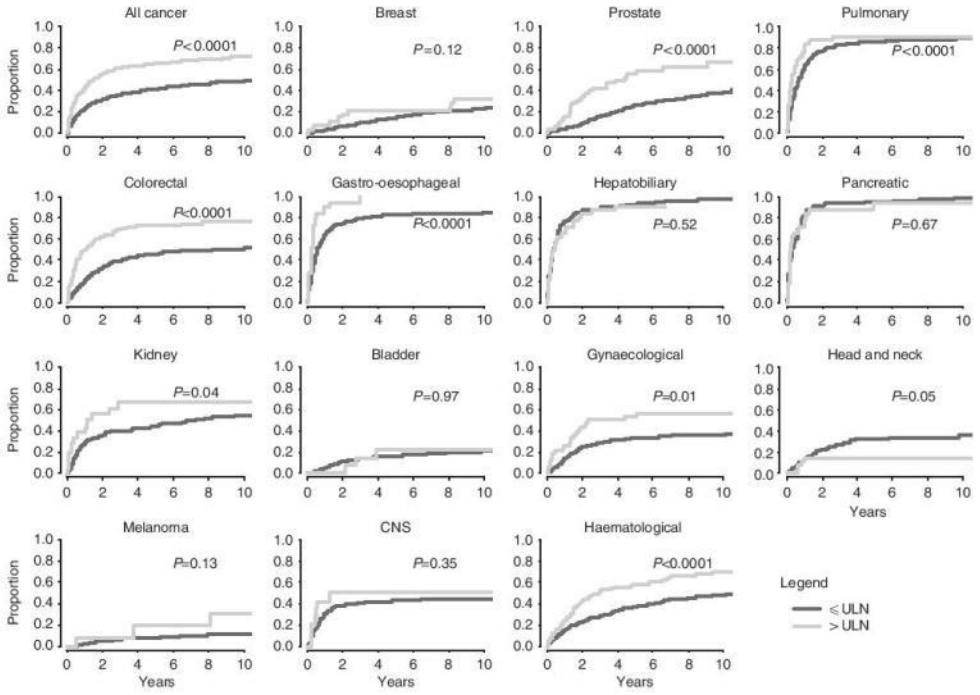

Figure 3. The 10-year cumulative incidence of cancer-specific deaths, stratified by clinical categories of serum LDH. CNS = central nervous system.

We observed a borderline inverse trend between LDH and head and neck cancer death, although our analysis was limited by the number of events. The unique distribution of LDH subunits (Augoff *et al*, 2014) may explain different associations of serum expression of LDH with specific cancer types. Supporting this notion, our observation of average LDH levels measured by

Table 3. Hazard ratios and 95% confidence intervals for temporal associations between serum LDH and risk of death following cancer diagnosis

		LDH[a]		
Lag time,[b] months	No. of deaths/all patients	Z-score	⩽ULN	>ULN
All-cause death				
0–6	1952/2426	1.14 (1.11–1.17)	1.0 (Ref)	2.01 (1.79–2.25)
6–12	1124/1523	1.10 (1.03–1.18)	1.0 (Ref)	1.48 (1.21–1.83)
12–18	1188/1620	1.04 (0.96–1.12)	1.0 (Ref)	1.01 (0.79–1.31)
18–24	1176/1589	1.10 (1.02–1.18)	1.0 (Ref)	1.23 (0.96–1.57)
24–30	1287/1750	1.04 (0.96–1.13)	1.0 (Ref)	1.16 (0.90–1.51)
30–36	1300/1818	1.10 (1.01–1.19)	1.0 (Ref)	1.46 (1.15–1.86)
Cancer-specific death				
0–6	1498/2426	1.15 (1.12–1.18)	1.0 (Ref)	2.11 (1.86–2.40)
6–12	801/1523	1.11 (1.02–1.20)	1.0 (Ref)	1.54 (1.21–1.96)
12–18	839/1620	1.04 (0.95–1.15)	1.0 (Ref)	1.08 (0.80–1.47)
18–24	837/1589	1.07 (0.98–1.17)	1.0 (Ref)	1.07 (0.79–1.46)
24–30	913/1750	0.99 (0.90–1.10)	1.0 (Ref)	0.97 (0.68–1.38)
30–36	906/1818	1.04 (0.94–1.15)	1.0 (Ref)	1.10 (0.80–1.52)

Abbreviations: LDH = lactate dehydrogenase; Ref = reference; ULN = upper limit of normal. All models were adjusted for age at diagnosis, sex, socioeconomic status, Charlson comorbidity index, period of diagnosis and stratified by cancer sites.
[a]Average of serum LDH measurements taken within each interval time.
[b]Interval time between baseline measurement of serum LDH and cancer diagnosis.

6-month intervals before diagnosis showed varying trends across different cancer sites that may indicate a different extent of aerobic glycolysis with respect to cancer types. It is known that the majority of LDH subunits detected in the serum is LDH-B, although LDH-A exists in a lesser amount (Maekawa, 1988). The absence of *LDH-B* expression and its enzyme activities have been reported in cell lines of breast (Brown *et al*, 2013), prostate (Leiblich *et al*, 2006), gastric and pancreatic cancer (Maekawa *et al*, 2003), suggested to be driven by promoter hypermethylation. As LDH-B kinetically favours the backward reaction of pyruvate–lactate conversion (Augoff *et al*, 2014), this may suggest that LDH-A, which mostly catalyses the formation of lactate, is more relevant to cancer than LDH-B (Maekawa, 1988). However, recent evidence has shown that higher tissue expression of LDH-B correlates to overall survival in lung cancer and treatment response in breast cancer (Dennison *et al*, 2013; McCleland *et al*, 2013), highlighting the role of LDH-B in cancer progression. Given the scarcity of data regarding the long-term impact of differential LDH expression on cancer survival, further investigations are needed to confirm the clinical usefulness of LDH with respect to its subunits or isoenzymes.

In addition to the positive association between prediagnostic LDH and death following cancer diagnosis, we were able to demonstrate the importance of timing in LDH measurement. Lactate dehydrogenase measured within 12 months before the diagnosis of cancer was shown to be strongly associated with overall and cancer-specific death, further indicating the relevance between LDH and tumour growth or severity. The positive association between LDH measured within 30 to 36 months before diagnosis and risk of overall as well as breast cancer death further signifies the importance of assessing cancer-specific death, especially because higher LDH is also linked to cardiovascular disease and mortality (Savory and Pryce, 1980; Kemp *et al*, 2004).

The strengths of our study included the prospectively collected serum LDH before the diagnosis of cancer. Complete follow-up was obtained and all laboratory measurements were performed in the same laboratory (Holme *et al*, 2010). Although a number of studies have indicated the association between LDH and overall survival (Petrelli *et al*, 2015), this is the first population-based study linking baseline LDH and cancer-specific survival. A limitation of our study is the lack of information on cancer treatment, and given the long period of recruitment (1986–1999), variation in management of cancer may affect timing of cancer diagnosis and its survival. We therefore accounted for period of diagnosis in our analyses as a proxy for difference in screening and treatment over time. Information on race/ethnicity was not available; however, the AMORIS cohort was similar to the general working population of Stockholm (Wulaningsih *et al*, 2013b) that comprised ~80% Swedish-born individuals in 2000 (Statistics Sweden, 2015). Serum LDH increases because of other conditions such as myocardial infarction, inflammation and tissue injury (Drent *et al*, 1996; Kemp *et al*, 2004; Kato *et al*, 2006), and therefore is not a specific marker of tumour. Higher LDH at baseline may otherwise indicate inflammation or other disorders involved in pathways leading to cancer development. However, we limited our analysis to 3 years before diagnosis to exclude reverse causation and adjusted for CCI in the analysis to take into account other diseases that may have predisposed one to worse survival. Nevertheless, residual confounding may occur. In addition, we did not have information on LDH subunits or isoenzymes and tumour characteristics such as stage, receptor status and histological grade. However, associations between LDH and all-cause or specific cancer death in breast cancer patients were not affected by tumour stage. For several cancers such as lymphoma, the combination between serum LDH and tumour characteristics shows to be useful in predicting treatment response and prognosis (Zhou *et al*, 2014). Thus, extrapolating our findings to clinic may require similar combination approaches with other biological markers in order to identify patients at higher risks of dying from cancer.

CONCLUSION

Based on prospectively collected serum LDH, our study demonstrated an inverse association between LDH and survival following cancer diagnosis, adding to the current evidence on the role of LDH in cancer progression. Future mechanistic studies are therefore necessary to establish whether serum LDH is a proxy of tumour growth and severity, which explains its association to cancer survival, or whether it is also involved in early carcinogenesis.

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CONFLICT OF INTEREST

Niklas Hammar is employed by AstraZeneca. However, the views expressed in this study are his own and not those of AstraZeneca. The remaining authors declare no conflict of interest.

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Appendix A7. A competing risks analysis of the association between prediagnostic serum glucose and lipids and breast cancer survival. Poster presented at the 2015 San Antonio Breast Cancer Symposium, December 2015. Awarded with an American Association for Cancer Research (AACR) Scholar-in-Training Award.

A competing risks analysis of the association between prediagnostic serum glucose and lipids and breast cancer survival

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San Antonio Breast Cancer Symposium - December 8-12, 2015

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Gunnar and Ingmar Jungner Foundation for Laboratory Medicine

Background and Aims

- Abnormal glucose and lipid metabolisms have been linked to breast cancer (BC) incidence but their impact on prognosis is unclear.
- Most studies focusing on BC survival employ survival analytical methods which assume non-informative censoring.
- Current problem: glucose and lipids are linked to cardiovascular (CV) disease → competing risks, informative censoring.
- We studied the associations of serum glucose and lipids with BC death while taking into account death from CV disease and other causes, thereby addressing the effect of competing risks.

Methods

- The Swedish Apolipoprotein Mortality Risk Study (AMORIS)
- A total of 1,798 women aged 20+ with diagnosis of BC diagnosis during 1985-1999
- Baseline serum glucose, triglycerides (TG) and total cholesterol (TC) measured 3 months to 3 years before diagnosis

Statistical Analysis

- Two analytical approaches: Cox regression and competing risk analysis with latent class proportional hazards model (**Figure 1**)

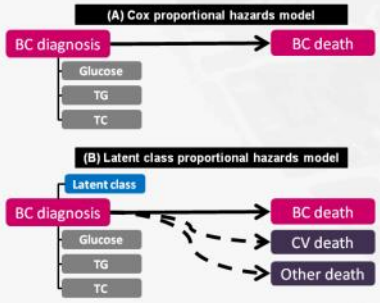

Figure 1. Schematic representation of analytical methods

Results

- A total of 861 (47.9%) patients died during a mean follow-up of 13 years: 425 from BC, 179 from CV disease, and 257 from other causes.
- Mean age at diagnosis: 58 years
- Higher proportion of deaths with higher levels of glucose, TG, and TC (**Figure 2**)

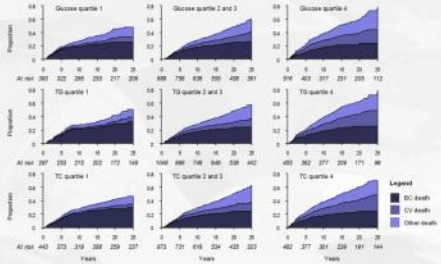

Figure 2. Stacked cumulative risk of death from BC, CV disease, and other causes, stratified by quartiles of glucose, TG and TC.

Table 1. Characteristics of participants by predicted class membership

	BC				
	Class I (N = 1,466)		Class II (N = 332)		P-value
	N	%	N	%	
Age, years					<0.0001
Mean (SD)	57.6 (10.9)		60.5 (15.0)		
Socioeconomic status					<0.0001
White collar	554	37.8	94	28.3	
Blue collar	739	50.4	155	46.7	
Unemployed or unknown	173	11.8	83	25.0	
Fasting status					0.55
Fasting	827	56.4	200	60.2	
Non-fasting	477	32.5	91	27.4	
Missing	162	11.1	41	12.4	
Glucose (mmol/l)					0.08
Mean (SD)	5.1 (1.3)		5 (1.1)		
TG (mmol/l)					0.32
Mean (SD)	1.3 (0.8)		1.3 (0.8)		
TC (mmol/l)					0.34
Mean (SD)	5.9 (1.2)		6.0 (1.2)		

Cox proportional hazards model

- No association between the three markers and BC death
- A positive association between TG and glucose and CV death

Latent class proportional hazards model

- Bayesian model selection indicated two latent classes (**Table 1**).
- Younger mean age in Class I (81.5%); socioeconomic status differed across classes.
- Higher mortality in Class II, with different association of the three markers with BC death and competing outcomes (**Figure 3**)

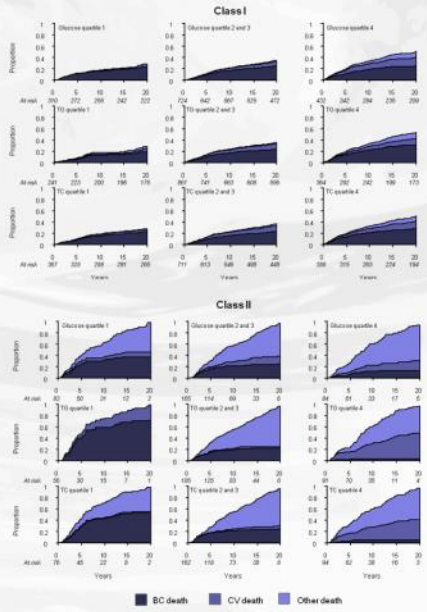

Figure 3. Stacked cumulative risk of death from BC, CV disease, and other causes for each latent class, stratified by quartiles of glucose, TG and TC.

Table 2. Hazard ratios (HR) and 95% confidence intervals (CI) of BC death by predicted class membership

	Class I		Class II	
	HR	95% CI	HR	95% CI
BC death				
Log glucose	1.09	0.73, 1.63	0.84	0.45, 1.57
Log TG	1.87	1.01, 3.45	0.91	0.50, 1.68
Log TC	0.84	0.49, 1.45	1.02	0.53, 1.99
CV death				
Log glucose	1.02	0.55, 1.91	1.46	0.97, 2.20
Log TG	7.68	2.45, 24.02	0.71	0.40, 1.25
Log TC	0.86	0.32, 2.28	2.07	1.16, 3.69
Other death				
Log glucose	0.73	0.50, 1.05	2.26	1.50, 3.40
Log TG	1.69	0.95, 3.01	1.40	0.74, 2.64
Log TC	1.20	0.65, 2.24	0.45	0.19, 1.06

- Higher TG corresponded with an increased risk of dying from BC in Class I (HR: 1.87, 95% CI: 1.01-3.45 for every increased in log-transformed TG).
- TC was positively associated with death from CV disease, and glucose with death from other causes in Class II patients (**Table 2**).

Conclusion

- A weak positive association between serum TG and risk of dying from BC among the majority of BC patients → pathways involved in perturbed lipid metabolisms may play a role in BC progression.
- There was marked heterogeneity of associations of serum glucose and lipids with BC death in presence of competing outcomes.
- Our findings highlight cohort heterogeneity and risk correlations in assessment of BC survival as an important yet understudied subject of investigations.

→Potential impact on breast cancer risk stratification and prognosis

Appendix A8. Prediagnostic serum glucose and lipids in relation to survival in breast cancer patients: a competing risk analysis. Published in BMC Cancer, 2015.

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Prediagnostic serum glucose and lipids in relation to survival in breast cancer patients: a competing risk analysis

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Abstract

Background: Abnormal glucose and lipids levels may impact survival after breast cancer (BC) diagnosis, but their association to other causes of mortality such as cardiovascular (CV) disease may result in a competing risk problem.

Methods: We assessed serum glucose, triglycerides (TG) and total cholesterol (TC) measured prospectively 3 months to 3 years before diagnosis in 1798 Swedish women diagnosed with any type of BC between 1985 and 1999. In addition to using Cox regression, we employed latent class proportional hazards models to capture any heterogeneity of associations between these markers and BC death. The latter method was extended to include the primary outcome (BC death) and competing outcomes (CV death and death from other causes), allowing latent class-specific hazard estimation for cause-specific deaths.

Results: A lack of association between prediagnostic glucose, TG or TC with BC death was observed with Cox regression. With latent class proportional hazards model, two latent classes (Class I and II) were suggested. Class I, comprising the majority (81.5 %) of BC patients, had an increased risk of BC death following higher TG levels (HR: 1.87, 95 % CI: 1.01–3.45 for every log TG increase). Lower overall survival was observed in Class II, but no association for BC death was found. On the other hand, TC positively corresponded to CV death in Class II, and similarly, glucose to death from other causes.

Conclusion: Addressing cohort heterogeneity in relation to BC survival is important in understanding the relationship between metabolic markers and cause-specific death in presence of competing outcomes.

Keywords: Breast cancer, Glucose, Lipid, Competing risk, Survival, Latent class

Background

Disorders in glucose and lipid metabolism have been suggested as a mechanism linking obesity and breast cancer (BC) [1, 2]. In addition to their roles in carcinogenesis, increasing evidence suggests that abnormal levels of serum glucose and lipids impact survival in BC patients [3–5]. Most of these studies investigated all-cause mortality as the outcome of interest. When BC-specific death is studied as the primary outcome, information on other causes of death such as cardiovascular (CV) disease is rarely addressed in the analysis [4]. Given the high survivorship of BC [6, 7] and how glucose and lipids are linked to CV mortality [8, 9], one must consider the possibility of competing risks. For instance, a competing risk situation arises when a person has a common risk factor of dying from both BC and CV disease (and other causes), so that any earlier outcome will 'prevent' the individual from developing others [10]. Interpreting survival data thus becomes difficult because commonly used methods, i.e., Kaplan-Meier survival estimates and Cox' proportional hazards, rely on the

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assumption of non-informative censoring. When this assumption is met, any censoring due to non-primary events does not affect one's risk of developing the primary outcome, thus such a risk is proportional to the levels of risk factors or covariates observed. However, when competing risks are an issue a heterogeneous association between covariates and the primary outcome may exist, reflecting subpopulations or classes with different mortality risk profiles. This heterogeneity within a cohort is scarcely studied in the context of cancer survival.

The objectives of the present study were to investigate how prediagnostic serum glucose, triglycerides (TG) and total cholesterol (TC) are associated to BC death, and to capture heterogeneity of associations between these markers and BC death which may indicate a competing risk situation. We used prospectively collected data from the Apolipoprotein Mortality Risk (AMORIS) Study and utilised 1) Cox proportional hazards model to assess the link between serum glucose, TG and TC with BC death, and 2) latent class proportional hazards models with BC death as the primary outcome and deaths from CV disease and other causes as non-primary outcomes to capture heterogeneity of BC mortality risk.

Methods

Study population

The Apolipoprotein Mortality Risk (AMORIS) Study has been described in detail elsewhere [11, 12]. Briefly, the recently updated AMORIS database comprises 812,073 individuals with blood samples sent for laboratory testing to the Central Automation Laboratory (CALAB) in Stockholm, Sweden. Individuals recruited were mainly from the greater Stockholm area, and either healthy and having laboratory testing as a part of general check-up, or outpatients referred for laboratory testing. None of the participants were inpatients at the time the samples were analysed. In the AMORIS study, the CALAB database was linked to Swedish national registries such as the Swedish National Cancer Register, the Hospital Discharge Register, the Cause of Death Register, the consecutive Swedish Censuses during 1970–1990, and the National Register of Emigration using the Swedish 10-digit personal identity number, providing complete follow-up information until 31 December 2011.

From the AMORIS population, we selected 1798 women with an incident diagnosis of BC between 1985 and 1999 who had baseline measurements of serum glucose, TG and TC within 3 months to 3 years prior to diagnosis. Diagnosis of BC was obtained from the Swedish National Cancer Register using the Seventh Revision of the International Classification of Diseases code (ICD-7 code: 174), and information on cause-specific deaths (BC death, CV death) was obtained from the Swedish Cause of Death Register. Follow-up time was defined as the time from diagnosis until death from any causes, emigration, or end of study (31 December 2011), whichever occurred first. The ethics review board of the Karolinska Institute approved the study, and permits were obtained from Swedish Data Inspection to correlate laboratory results with Swedish national registers. Anonymity of participants was maintained throughout the study. Participant informed consent was not required for this register linkage study [13].

Serum glucose and lipids measurements

Serum levels of glucose (mmol/L), TG (mmol/L), and TC (mmol/L) were measured enzymatically with standard methods [12]. All three markers were measured at the same day, within 3 months to 3 years prior to diagnosis. This timeframe was selected to capture metabolic derangements during ongoing malignancy process while excluding effects of breast cancer diagnostic or treatment interventions. All measurements were fully automated with automatic calibration and performed at one accredited laboratory [11]. TG levels were not normally distributed, and therefore we used log-transformed values of all markers in addition to their quartiles in the analysis.

Covariates

Information on fasting status at baseline measurements (fasting, non-fasting, unknown) was obtained from the CALAB database. Socioeconomic status (SES; white collar, blue collar, unemployed or unknown) was based on occupational groups in the Population and Housing Census and classified all gainfully employed subjects as manual workers and non-manual workers, which were referred to as blue collar and white collar workers, respectively [14].

Statistical analysis

We began by employing multivariable Cox proportional hazards regression to assess the association between log-transformed values and quartiles of glucose, TG and TC and the risk of BC death as the primary outcome, CV death and other death as competing outcomes. Adjustment was performed for potential confounders including age at diagnosis, SES, and fasting status at baseline measurements. Glucose, TG and TC were each analysed while adjusting for the other two markers as continuous variables. The proportionality of hazards assumption was met after assessing time-varying covariates which were the cross-products of each variable and time. To assess any potential competing risk, we used cumulative incidence functions to display the proportions of deaths from BC, CV disease and other causes by quartiles of glucose, TG, and TC.

We further investigated the association between serum glucose, TG and TC and BC survival using a latent class proportional hazards model. Latent class analysis has been used to identify different classes or latent variables within a given population which underlies the pattern of association between observed covariates [15]. In medical research, the latent class variable has been incorporated into various regression analyses, including Cox proportional hazards models, to allow identification of subgroups with different risk profiles [16–18]. To capture heterogeneity in the context of BC survival, we extended the proportional hazards model to encompass the latent class variable in addition to glucose, TG and TC, which were assessed as continuous variables. The number of latent classes present in the cohort was identified with Bayesian model selection. To assess BC-specific death whilst accounting for competing risks, we incorporated BC death as the primary outcome and deaths from CV disease and other causes as non-primary outcomes into the latent class proportional hazards model. Class membership probabilities were retrospectively predicted based on associations between covariates and events. Independent samples *T*-test and Chi^2 test were used to assess differences in characteristics of study participants by predicted class membership. We further displayed latent class-specific cumulative incidence functions for BC, CV and other death by quartiles of the three markers. Finally, hazard ratios for BC, CV and other death by levels of glucose, TG, and TC were estimated for each latent class according to the maximum-a-posteriori (MAP) likelihood, which took into account all three outcomes [19]. More details on the latent class survival analysis are available as Additional file 1.

Descriptive analysis and Cox proportional hazards model were performed with Statistical Analysis Software (SAS) release 9.3 (SAS Institute, Cary, NC) and R

Table 1 Descriptive characteristics of study participants overall and by causes of death

	All BC		Overall death		BC death		CV death		Other death	
	(*n* = 1798)		(*n* = 861)		(*n* = 425)		(*n* = 179)		(*n* = 257)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Age, years										
Mean	58.1		62.4		56.5		71		66.2	
SD	11.8		13.2		12.5		10.3		11.4	
Follow-up time, years										
Mean	13.3		8.3		6.4		9.3		10.6	
SD	6.9		5.9		5.0		6.5		6.0	
Interval between measurements and diagnosis, months										
Mean	18.3		18.1		18.3		17.6		17.9	
SD	9.2		9.2		9.0		9.5		9.2	
SES										
White collar	648	36.0	235	27.3	147	34.6	30	16.8	58	22.6
Blue collar	894	49.7	405	47.0	222	52.2	61	34.1	122	47.5
Unemployed or unknown	256	14.3	221	25.7	56	13.2	88	49.1	77	29.9
Fasting status										
Fasting	1027	57.1	508	59.0	242	56.9	107	59.7	159	62.9
Non-fasting	568	31.6	254	29.5	133	31.3	52	29.1	69	26.8
Unknown	203	11.3	99	11.5	50	11.8	20	11.2	29	11.3
Glucose, mmol/L										
Mean	5.1		5.2		5.0		5.5		5.4	
SD	1.2		1.4		1.0		1.2		1.8	
TG, mmol/L										
Mean	1.3		1.4		1.3		1.6		1.4	
SD	0.8		0.9		0.9		0.9		0.8	
TC, mmol/L										
Mean	5.9		6.1		5.9		6.5		6.2	
SD	1.2		0.8		1.2		1.2		1.2	

version 3.0.2 (R Project for Statistical Computing, Vienna, Austria). Latent class proportional hazards model were performed with Advanced Survival Analysis software version 0.2.16 (A.C.C. Coolen, M. Rowley, M. Inoue, London, UK).

Results

At the end of follow up (mean: 13 years), a total of 861 (47.9 %) study participants were deceased. Among these women, 425 died from BC, 179 from CV disease, and 257 from other causes. The mean age of all participants was 58 at BC diagnosis. Levels of glucose, TG, and TC were highest in those dying from CV disease, whereas women who died from BC had lower levels of the three markers compared to all women dying during follow-up period (Table 1).

When conventional Cox proportional hazards regression was performed, no strong association was observed between glucose, TG, and TC and risk of dying from BC (Table 2). On the other hand, positive associations were observed between TG and CV death, as well as glucose and CV death. No association was observed for other causes of death. Proportions of deaths from each causes by quartiles of glucose, TG, TC was further displayed using the cumulative incidence functions. As shown in Fig. 1, the proportion of women dying from CV disease markedly increased with higher quartiles of the markers, whilst deaths from BC are less frequent with higher quartiles of the markers. This indicated CV death as a competing event.

Our next analysis extended the proportional hazards model to include latent class variables and assess primary and non-primary outcomes. Bayesian model

Table 2 Hazard ratios of death from BC, CV disease and other causes by levels of glucose, TG, and TC

	No. of subjects	BC death			CV death			Other death		
		No. of events	HR[a]	95 % CI	No. of events	HR[a]	95 % CI	No. of events	HR[a]	95 % CI
Glucose, mmol/L[b]										
Continuous log			0.96	0.58, 1.59		2.48	1.24, 4.96		2.09	1.16, 3.76
Quartiles										
< 4.50	393	98	1		21	1		45	1	
4.50–4.90	413	116	0.98	0.75, 1.29	36	1.27	0.74, 2.19	63	1.12	0.76, 1.64
4.90–5.30	363	96	0.95	0.72, 1.27	41	1.28	0.75, 2.19	50	0.87	0.58, 1.30
≥ 5.30	416	115	0.98	0.74, 1.29	80	1.67	1.02, 2.73	100	1.32	0.92, 1.89
P_{trend}			0.83			0.03			0.20	
TG, mmol/L[c]										
Continuous log			1.21	0.98, 1.48		1.58	1.17, 2.13		1.32	1.02, 1.71
Quartiles										
< 0.70	297	81	1		12	1		24	1	
0.70–1.00	491	102	0.77	0.57, 1.04	34	0.91	0.46, 1.77	56	0.96	0.59, 1.57
1.00–1.60	555	132	0.97	0.72, 1.29	52	1.10	0.58, 2.08	95	1.28	0.81, 2.03
≥ 1.60	455	110	1.05	0.76, 1.45	80	1.53	0.81, 2.90	83	1.22	0.75, 1.98
P_{trend}			0.35			0.01			0.16	
TC, mmol/L[d]										
Continuous log			0.72	0.40, 1.28		2.04	0.83, 5.04		0.67	0.32, 1.42
Quartiles										
< 5.20	443	119	1		16	1		38	1	
5.20–5.80	403	94	0.87	0.66, 1.14	37	1.52	0.83, 2.76	60	1.18	0.78, 1.79
5.80–6.60	470	102	0.79	0.60, 1.04	40	1.26	0.70, 2.27	75	1.06	0.72, 1.58
≥ 6.60	482	110	0.85	0.64, 1.15	85	1.74	0.99, 3.04	85	0.92	0.61, 1.38
P_{trend}			0.21			0.08			0.38	

[a]Adjusted for age at diagnosis, SES (white collar, blue collar, unemployed or unknown), fasting status (fasting, non-fasting, unknown), glucose (continuous), TG (continuous), and TC (continuous)
Not adjusted for [b]glucose, [c]TG, [d]TC

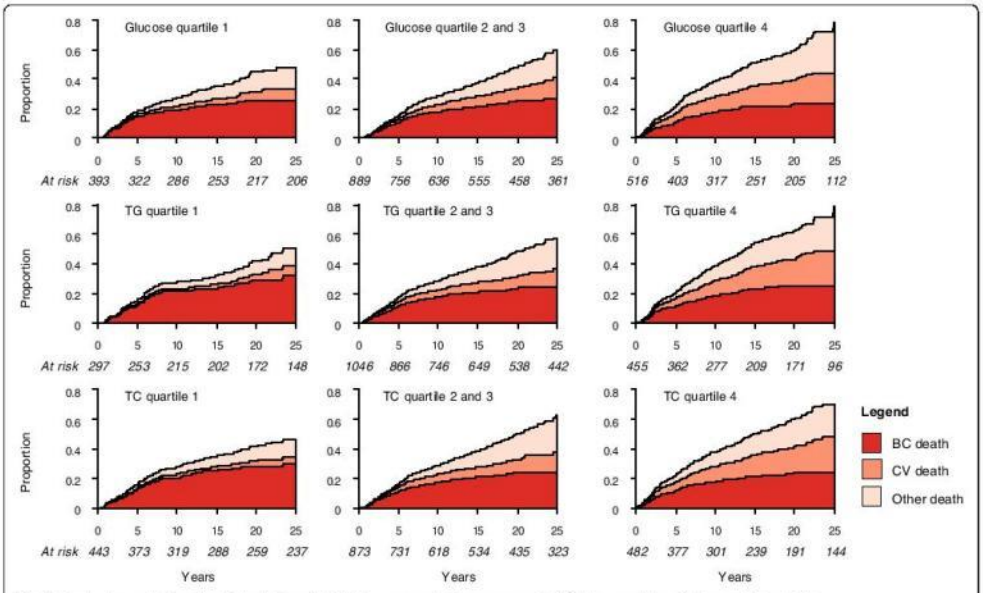

Fig. 1 Stacked cumulative risk of death from BC, CV disease, and other causes, stratified by quartiles of glucose, TG and TC

selection identified two latent classes in this study population. Retrospective analysis for class membership probability suggested that 81.5 % women were more likely to be members of Class I, while the other 18.5 % belonged to Class II. We further assessed baseline characteristics of study participants in relation to the most probable latent class they were assigned to. Younger average age was observed in Class I compared to Class II, and a difference in socio-economic status between classes was indicated (Table 3). With regards to clinical outcomes, no difference in proportions of women who died from BC was found between the two classes. However, statistically significantly higher overall mortality rate from CV disease and other causes were seen in Class II.

We further investigated difference in survivals between latent classes by displaying cumulative incidence functions for different causes of death by quartiles of glucose, TG, and TC (Fig. 2). Higher overall mortality was seen in Class II compared to Class I. In Class I, most patients died from BC, whereas in Class II, most died from other causes apart from BC and CV death. Increasing absolute numbers of deaths from BC, CV, and other causes were seen with higher levels of all three markers in Class I, although there was no marked difference in relative mortality rates between each cause of death. On the other hand, marked differences in relative proportions of women dying from the three different causes were seen across levels of markers in Class II. For instance, BC deaths were common amongst women in the lowest quartiles of glucose, TG, and TC, but contributed little to total deaths in those with highest levels of the markers. More women died from CV disease with higher TC, and a similar association was seen between glucose and death from other causes. Finally, the risk of different causes of death was quantitatively assessed by obtaining class-specific hazard estimates. As seen in Table 4, log-transformed TG corresponded to an increased risk of dying from BC in Class I, with a hazard ratio of 1.87 (95 % CI: 1.01–3.45). No statistically significant associations with BC death were observed for other markers or among women in Class II. In agreement with class-specific cumulative incidence functions, women in Class II had a higher risk of CV death with higher TC and a higher risk of other death with higher glucose levels.

Discussion

We performed Cox regression and a latent class proportional hazards analysis to assess the association between prediagnostic markers of glucose and lipid metabolism and death from BC in female BC patients. The latter method accounted for CV death and other death as competing risks. With the conventional Cox

Table 3 Characteristics of study participants and causes of death by predicted class membership

	BC				P-value
	Class I (*N* = 1466)		Class II (*N* = 332)		
	N	%	N	%	
Age, years					<0.0001
Mean	57.6		60.5		
SD	10.9		15.0		
SES					<0.0001
White collar	554	37.8	94	28.3	
Blue collar	739	50.4	155	46.7	
Unemployed or missing	173	11.8	83	25.0	
Fasting status					0.55
Fasting	827	56.4	200	60.2	
Non-fasting	477	32.5	91	27.4	
Missing	162	11.1	41	12.4	
Glucose (mmol/l)					0.08
Mean	5.1		5		
SD	1.3		1.1		
TG (mmol/l)					0.32
Mean	1.3		1.3		
SD	0.8		0.8		
TC (mmol/l)					0.34
Mean	5.9		6.0		
SD	1.2		1.2		
BC death	342	23.3	83	25.0	0.52
CV death	129	8.8	50	15.1	<0.0001
Other death	60	4.1	197	59.3	<0.0001

proportional hazards model, a lack of association was observed between the three markers and BC death. However, CV death was shown as a competing event. When latent class proportional hazards analysis were performed, we found two distinct latent classes within our cohort, reflecting different susceptibilities of dying from BC based on their baseline characteristics. Class I, comprising the majority of the study population, is associated with an increased risk of BC death following higher TG levels. Overall survival is worse in Class II, among which higher TC levels were associated with an increased risk CV death and higher glucose with risk of death from other causes. No association between the three markers and BC death was seen in Class II.

Metabolisms of glucose and lipid have been implicated in many chronic diseases. In the context of cancer, an array of evidence has linked increased BC incidence with aberrant levels of circulating glucose, TG and TC at baseline [20–22]. Abnormal levels of these markers are also associated with CV disease, which is the most common cause of death in general population [8, 9]. This has also been demonstrated in our study, as both glucose and TG were associated with a higher risk of CV death, and the associations were stronger than those with BC death. Several biological mechanisms are suggested to underlie this common link, such as chronic inflammation and insulin resistance, which may drive atherogenesis, cellular proliferation and angiogenesis [2, 23, 24]. These shared metabolic pathways may thus result in a competing risks situation, where individuals with similar sets of risk factors are equally at risk of dying from both BC and CV disease. In this case, a heterogeneous association between glucose and lipid markers and BC death may be observed, which represents subpopulations or latent classes with different mortality risk profiles. However, this heterogeneity in survival data is not addressed by common analytical methods in cancer epidemiology.

Cox proportional hazards regression and latent classes proportional hazards model differ fundamentally in the assumptions made regarding risk correlations. In Cox, non-informative censoring is assumed, which leads to the assumption of independence or no correlation between event times when multiple events are observed. However, in the real-world clinical observation, such assumptions are rarely assessable and sometimes inaccurate. The latent class proportional hazards model allows for the presence of heterogeneity underlying any observed risk associations [16] and predicts optimal parameters based on the most probable substructure of the study population. In our study, this resulted in an optimal model with two latent classes. Overall survival was lower in Class II than Class I, which indicates the importance of taking into account risk associations when investigating biological markers in relation to cancer survival.

We found TG to be associated with early death from BC in Class I. This suggests an importance of lipid metabolism in disease progression in a relevant subset of BC patients, which warrants further mechanistic investigation. No statistically significant association with BC death was observed for glucose and TC, although among Class II they were associated with higher risks of dying from other causes and CV disease, respectively. Previous studies have reported a null association for TG and TC in relation to all-cause mortality [25] and BC-specific death [26], which is similar to our findings using Cox regression and in Class II as assessed by latent classes proportional hazards model. Likewise, a lack of association with overall death has been reported for glucose [4, 5]. Although Class I comprised the majority of all women

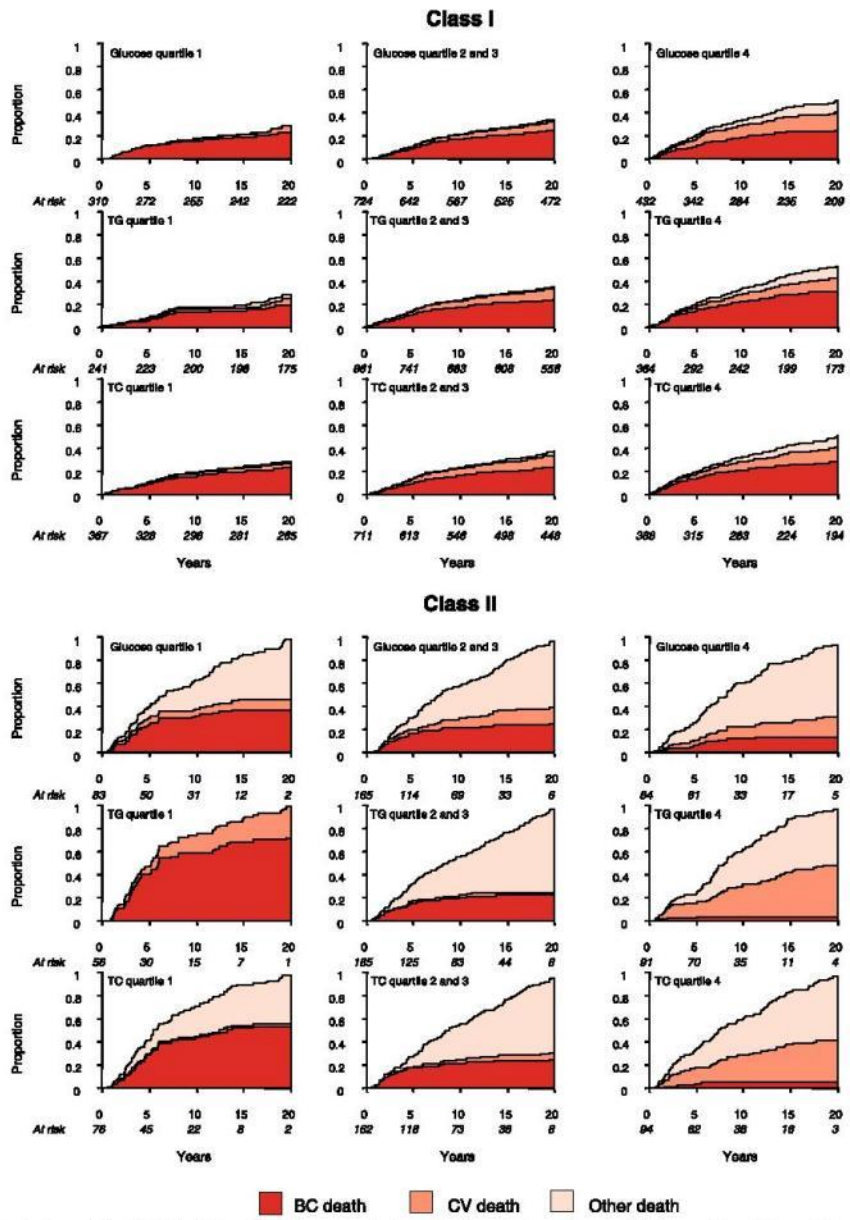

Fig. 2 Stacked cumulative risk of death from BC, CV disease, and other causes for each latent class, stratified by quartiles of glucose, TG and TC

Table 4 Hazard ratios of death from BC, CV disease and other causes by levels of glucose, TG, and TC for each latent class

	Class I		Class II	
	HR[a]	95 % CI	HR[a]	95 % CI
BC death				
Log glucose	1.09	0.73, 1.63	0.84	0.45, 1.57
Log TG	1.87	1.01, 3.45	0.91	0.50, 1.68
Log TC	0.84	0.49, 1.45	1.02	0.53, 1.99
CV death				
Log glucose	1.02	0.55, 1.91	1.46	0.97, 2.20
Log TG	7.68	2.45, 24.02	0.71	0.40, 1.25
Log TC	0.86	0.32, 2.28	2.07	1.16, 3.69
Other death				
Log glucose	0.73	0.50, 1.05	2.26	1.50, 3.40
Log TG	1.69	0.95, 3.01	1.40	0.74, 2.64
Log TC	1.20	0.65, 2.24	0.45	0.19, 1.06

[a]All covariates were included in a single model and adjusted for age at diagnosis, SES (white collar, blue collar, unemployed or unknown) and fasting status (fasting, non-fasting, unknown)

studied, it is possible that the positive association between TG and Class I was diluted in the overall cohort, resulting in a weaker association. Therefore, it is important to consider cohort heterogeneity in assessing this relationship.

The strength of this study lies in the survival analysis method used to address competing risks, as well as the relatively large cohort with follow-up information for all participants (up to 25 years). The population in the AMORIS study was selected by analysing blood samples from health check-ups in non-hospitalised persons. However, any healthy cohort effect would not affect the internal validity of our study [11]. To our knowledge, this is the first observational study utilising latent class proportional hazards model to address disease-specific survival in BC, taking into account CV death and other death as competing events. As shown in our study, the advantage of incorporating latent class analysis and multiple events in addition to proportional hazards regression is that it allows identification of subpopulations within the cohort and final survival or hazard estimates of the primary event. In other words, this method may offer a suitable approach when dealing with survival functions or hazard rates estimation in presence of competing risks. A limitation of our study was the lack of data representing older BC patients, which may partly explain the low proportion of Class II. There was no information available on tumour characteristics, BC susceptibility genes, and treatment or other metabolic and endocrine factors related to BC such as obesity and use of hormonal replacement therapy. Although residual associations with unobserved covariates were captured by our model through identification of latent classes, underlying characteristics of these different subgroups of BC patients may require further integration of other relevant markers or baseline information.

Conclusion

The present study showed a weak association between prediagnostic TG levels and BC death in the majority of women with BC. On the other hand, glucose and TC were strongly associated to mortality from causes apart from BC in the remaining patients, among which shorter overall survival was observed. Our study therefore demonstrated heterogeneity in the association between glucose, lipid markers, and BC survival when CV death and other death were taken into account as competing outcomes. This implies an involvement of perturbed lipid metabolism in BC progression and a complex interaction between baseline biological markers and co-morbidities in determining BC survival which warrants mechanistic investigations. Therefore, our findings highlight the importance of considering cohort heterogeneity when evaluating biological markers in relation to cause-specific death.

Additional file

Additional file 1: Bayesian Survival Analysis with a latent class model. (DOC 37 kb)

Competing interest
The authors declare that they have no competing interests. Niklas Hammar is employed by the AstraZeneca, but the views expressed in the manuscript are his own and not those of AstraZeneca.

Authors' contributions
WW, MV, LH, HG and MVH conceived and designed the study. WW, LH, HG, ML, NH, GW, IJ, and MVH were responsible for data acquisition and quality control. WW, MV, MR, and ACC performed all data analysis. All authors interpreted study findings, prepared the manuscript and reviewed the final draft. All authors read and approved the final manuscript.

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Appendix B. Other Relevant Publications

Appendix B1. Serum leptin, C-reactive protein, and cancer mortality in the NHANES III. Published in Cancer Medicine, 2015.

Cancer Medicine

Open Access

ORIGINAL RESEARCH

Serum leptin, C-reactive protein, and cancer mortality in the NHANES III

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Abstract

Adipokines, such as leptin, may affect cancer through its link with inflammation and obesity. We investigated the association between leptin, C-reactive protein, and risk of cancer death while accounting general and abdominal obesity. From the Third National Health and Examination Survey (NHANES III), we selected 5957 adult men and women with baseline measurements of serum leptin and CRP. Multivariable Cox regression was used to assess leptin and CRP levels (low, moderate, high) in relation to risk of cancer death. Stratification analyses were performed for obesity as defined by body mass index (BMI) and waist circumference. Fine and Gray regression was performed to account for death from cardiovascular disease and other causes as competing events. A total of 385 participants died of cancer during a mean follow-up of 18 years. After adjusting for BMI and waist circumference, an inverse association with log-transformed leptin was found for women, with a hazard ratio (HR) of 0.81 (95% confidence interval [CI]: 0.51–1.30) and 0.40 (95% CI: 0.24–0.68) for moderate and high compared to low levels of leptin, respectively; $P_{trend} = 0.0007$). No association for leptin was observed in men, but higher CRP corresponded to increased risk of dying from cancer (HR: 2.98; 95% CI: 1.57–5.64 for the highest vs. lowest categories of CRP). Similar associations were observed with competing risk analysis also adjusted for BMI and waist circumference. Contrasting associations of serum leptin and CRP with cancer mortality may indicate sex-specific biological or environmental pathways linking obesity and cancer in men and women which warrant mechanistic investigations.

Introduction

Leptin is one of the most important hormones secreted by the adipocytes [1]. Besides regulating food intake and energy expenditure [2], leptin plays an essential role in hematopoiesis, reproductive function, and glucose and lipid metabolism [3]. More recently, leptin has also been linked to cancer [4]. Enhanced expressions of leptin and its receptor (Ob-R) are found in solid cancers including breast and ovarian cancers, and have been associated to metastasis and poor prognosis [5, 6]. However, conflicting evidence exists in the context of cancer incidence. For instance, in a meta analysis comprising 23 case–control studies, a protective effect of serum leptin against postmenopausal breast cancer was reported [7]. In contrast, a positive association was seen in recent nested case–control studies, where serum leptin was measured prospectively prior to diagnosis in breast cancer cases [8, 9]. Meanwhile, circulating Ob-R has been linked to a lower risk of colorectal cancer in a nested case–control study despite a null finding for leptin [10]. These inconsistent findings may reflect an involvement of other factors in the relationship between leptin and carcinogenesis, as well as potential time-sensitivity of this association.

Obesity may promote the development of cancer [11], but their mechanistic association remains unclear. There is indication that chronic inflammation may mediate obesity and cancer [12]. Interestingly, a role of leptin in inflammation has been suggested [13], as shown by a linear association between leptin and markers of inflammation [14]. Both increased inflammatory activity and leptin production are common features of obesity [15], thus it remains unclear whether pathways linking obesity and the development of cancer involve leptin production or inflammation, or whether there are simultaneous effects of these two processes on cancer susceptibility.

Presently, there is lack of observational studies assessing leptin in relation to cancer while accounting for inflammation and different definitions of obesity. Therefore, we sought to disentangle this complex association between leptin, inflammation and cancer by assessing serum levels of leptin and C-reactive protein (CRP), a common inflammatory marker [16], in relation to cancer mortality in the Third National Health and Nutrition Examination Survey (NHANES III) while accounting for general and abdominal obesity. Additionally, since both markers are linked to death from cardiovascular disease [17], we used cardiovascular mortality as a competing outcome in our analysis.

Methods

Study population

The National Center for Health Statistics (NCHS) conducted NHANES III between 1988 and 1994 and designed it as a multistage stratified, clustered probability sample of the U.S. civilian noninstitutionalized population who was at least 2 months old. All subjects participated in an interview conducted at home and an extensive physical examination, which included a blood sample taken in a mobile examination center [18]. Despite a cross-sectional design, mortality follow-up was provided by the NCHS through December 31, 2011, allowing the use of the dataset as a prospective cohort [19]. From recruited NHANES III participants, we selected 5957 men and women aged 20 and over who had baseline measurements of serum leptin and CRP, available information on body mass index (BMI) and waist circumference, and for whom follow-up information was available. No participant reported a history of any cancer at the baseline interview. The protocols for the conduct of NHANES III were approved by the Institutional Review Board of the NCHS, Centers for Disease Control and Prevention. Written informed consent was obtained from all participants [18].

Serum leptin and CRP measurements

Serum specimens were stored at −70°C and went through at least one freeze–thaw cycle during a mean of 8 year of storage before leptin concentrations were measured. Serum leptin was measured by radioimmunoassay at Linco Research, Inc. (St Charles, MO) [20]. The minimum detectable concentration of the assay is 0.5 ng/mL. Within- and between-assays coefficients of variation were <5%. Levels of serum leptin were categorized into low, moderate, and high based on sex-specific tertiles [21], with cut-off points of 3.3 and 6.3 μg/L for men and 10.8 and 20 μg/L for women. Serum CRP was measured with an automated Behring Nephelometer Analyzer System (Behring Diagnostics, Inc, Somerville, NJ) [22]. Coefficients of variation ranged from 3.2 to 16.0% throughout data collection. Tests were repeated for specimens with results of >10 mg/L. Because levels of CRP below 2.2 mg/L were undetectable in the NHANES III, we used clinical cut-off points as previously described [16]: low (<2.2 mg/L), moderate (2.2–10 mg/L), and high (≥10 mg/L).

Covariates

Information on age (years), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, and other), cigarette smoking (never, former, and current smokers), alcohol consumption (never, up to once/week, 2–3 times/week, 4–6 times/week, daily or more), vigorous physical activity (yes, no), and self-reported history of cancer (yes, no) was collected during the interview. Socioeconomic status was estimated with poverty-to-income ratio (PIR), a ratio of total family income to the official poverty threshold at the family level. A PIR <1 indicated that income was less than the level of poverty. We categorized PIR in this study into <1, 1–2, and ≥2.

Obesity status

Body measurements were performed using standardized methods and equipment [23]. Weight was measured in pounds and automatically converted to kilograms with an electronic weight scale. Participants only wore underwear, disposable paper gowns, and foam rubber slippers. Standing height was measured with a fixed stadiometer to the nearest 1 mm. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Waist circumference was measured at the high point of the iliac crest at minimal respiration using a steel measuring tape to the nearest 1 mm [23]. General obesity (obese, not obese) was defined as having a BMI of 30 kg/m^2 or more [24]. Abdominal obesity (obese, not obese) was defined as waist circumference of >102 cm in men and >88 cm in women [25].

Mortality and follow-up

Information on dates and causes of death was obtained from data linkage of the NHANES dataset with the National Death Index (NDI). This linkage was performed by the NCHS through probabilistic matching with social security number, birth date, occupation, and other personal data, and confirmation with death certificate when possible [19]. Follow-up time was calculated from interview date/examination date until date of death or end of study (31 December, 2011), whichever came first. Underlying causes of death were based on ICD-9 codes through 1998 and on International Classification of Diseases, 10th version (ICD-10) codes for deaths occurring after 1998. In order to adjust for changes between the two coding systems, final cause of deaths occurring prior to 1999 were re-coded into comparable ICD-10-based underlying cause of death groups [19]. The primary outcome of this study was cancer-specific death (ICD-10: C00-C97). Only aggregate information on leading causes of death is available in the 2011 mortality follow-up, thus rendering analysis by specific cancer sites not possible. Death from major cardiovascular diseases (ICD-10: I00-I09, I11, I13, I20-I51, I60-I69) and other causes were assessed as competing outcomes.

Statistical analysis

Sampling weights for NHANES III were used to account for sampling variability and to adjust for differential probability of selection of persons [18]. Due to differential distribution of serum leptin in men and women, we performed our analysis for men and women separately. Cox proportional hazards regression was used to assess risk of cancer death by categories of CRP and leptin. A test for trend was conducted by using assignment to categories as an ordinal scale. First, we carried out our analyses using two multivariable models: the first was adjusted for age, race/ethnicity, PIR, tobacco smoking, alcohol consumption, and vigorous physical activity. The final model included BMI and waist circumference to account for the effects of obesity. A test for multiplicative interaction between leptin and CRP was performed based on the suggested correlation between the two variables [21]. To further elucidate potential effect modification by obesity [16], we stratified our analysis based on general obesity status while adjusting for waist circumference, and by abdominal obesity while adjusting for BMI. In addition to interaction between leptin and CRP, we also assessed the interaction of each marker with obesity status. Finally, since the association of both markers and cardiovascular death [17] may affect their impact on cancer mortality, we performed Fine and Gray regression with deaths from major cardiovascular diseases and other causes as competing outcomes. The Fine and Gray analysis has been used to predict cumulative incidence of primary outcome in presence of competing outcomes, which may have created a competing risks situation [26]. We treated categories of leptin and CRP as ordinal variables and adjusted the models for age, race/ethnicity, PIR, tobacco smoking, alcohol consumption, vigorous physical activity, BMI, and waist circumference. Statistical significance was defined as two-sided P-values <0.05. All analyses were conducted with SAS release 9.3 (SAS Institute, Cary, NC) and R version 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria).

Table 1. Weighted characteristics of study population by sex.

	Men (N = 2759)	Women (N = 3198)
Age (years) – Mean (SD)	42.64 (0.55)	43.81 (0.60)
Follow-up (years) – Median (IQR[1])	19.35 (17.53–20.95)	19.38 (17.65–20.94)
Race – Ethnicity (%)		
Non-Hispanic white	76.52	76.42
Non-Hispanic black	9.77	11.32
Mexican American	5.56	4.78
Other	8.16	7.48
Poverty-to-income ratio		
<1	16.34	19.89
1–2	19.09	19.97
≥2	64.57	60.14
Alcohol consumption (%)		
Never	35.09	51.69
Up to once/week	18.15	21.28
2–3 times/week	16.50	13.11
4–6 times/week	15.44	8.19
Daily or more	14.82	5.73
Smoking behavior (%)		
Never	36.36	55.53
Former	32.29	20.13
Current	31.35	24.34
Vigorous Physical activity (%)	11.47	8.48
Waist circumference (cm) – Mean (SD)	95.50 (0.48)	88.22 (0.47)
Body mass index (kg/m^2)		
<18.5	0.68	2.981
18.5–25	38.57	46.04
25–30	40.46	27.06
≥30	20.28	24.09
Cancer death (%)	4.72	5.15
Cardiovascular death (%)	6.34	4.80

[1]Interquartile range.

Results

During a mean follow-up of 18 years, a total of 385 participants died of cancer and 507 from major cardiovascular diseases. Table 1 showed weighted characteristics of study participants by sex. Overall, increased leptin levels were observed in men and women following higher categories of CRP, BMI, and waist circumference (Fig. 1),

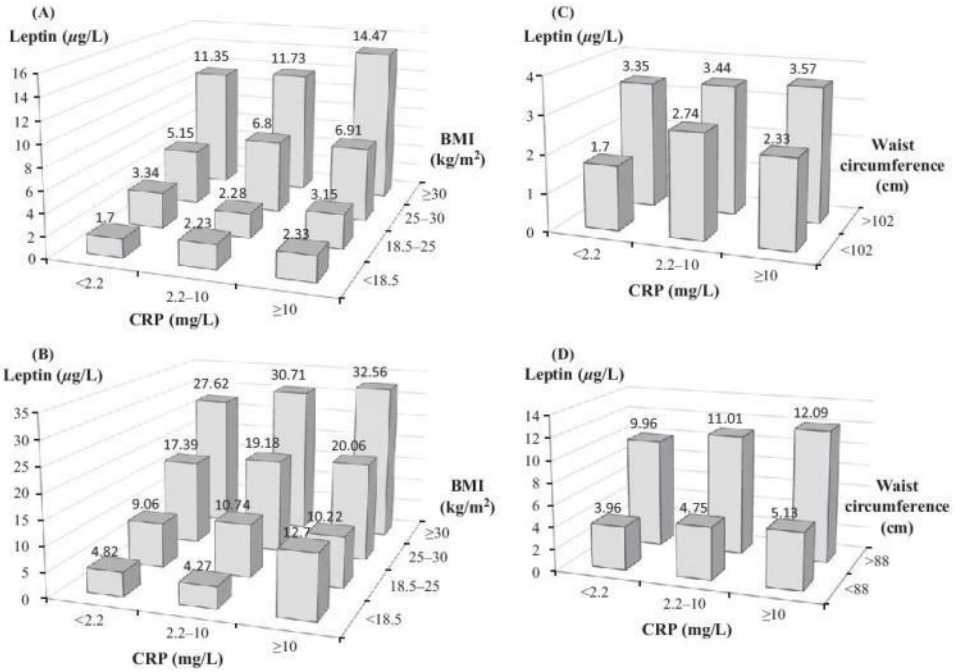

Figure 1. Serum concentrations of leptin by C-reactive protein (CRP) clinical cut-offs and body mass index in men (A) and women (B), and by CRP clinical cut-offs and waist circumference in men (C) and women (D).

with the highest concentrations of leptin seen in men and women with high CRP and BMI ≥ 30 kg/m^2 or the upper category of waist circumference.

When we examined the association between serum leptin or CRP and cancer death with the first model, high levels of CRP in men corresponded to higher risk of dying from cancer, with a hazard ratio (HR) of 2.98 and 95% confidence interval (CI) of 1.57 to 5.64 for the highest category of CRP compared to the lowest. No association was observed between leptin and cancer death in both sexes (Table 2). We further adjusted this model for obesity indicators, BMI and waist circumference, and no changes were seen with CRP. However, this revealed a marked inverse association between serum leptin and risk of cancer death in women (HR: 0.81; 95% CI: 0.51–1.30 and 0.40; 95% CI: 0.51–1.30 for moderate and high compared to low levels of leptin, respectively; P_{trend} = 0.0007). No interaction between categories of leptin and CRP was observed (Table 2).

We sought to further unpick the effect of obesity by stratification analyses based on general and abdominal obesity status. In men, no association between serum leptin and cancer death was observed (Table 3). For CRP, higher levels in men without general obesity was observed (HR: 3.62; 95% CI: 1.86–7.04 for high compared to low CRP, P_{trend} = 0.004). Interestingly, a higher risk of cancer death with the highest CRP levels was also seen in men with abdominal obesity (HR: 3.43; 95% CI: 1.03–11.48 compared to low CRP) but not those without. Nevertheless, no significant interaction between each definition of obesity and CRP was found. On the other hand, higher serum leptin was inversely associated with cancer death in women with or without general obesity, for example, HR among women with BMI <30 kg/m^2 was 0.76 (95% 0.44–1.32) and 0.30 (0.14–0.65) for moderate and high levels compared to low leptin, respectively (P_{trend} = 0.003). Results were less clear in stratification by waist circumference, or when CRP was assessed (Table 3). No strong interaction between leptin and CRP was suggested, although among men with abdominal obesity, interaction approached statistical significance (P = 0.07). Nevertheless, in a follow-up analysis when we included leptin and CRP in the same model, similar findings were observed.

Table 2. Sex-specific associations of serum leptin and C-reactive protein (CRP) with cancer death in the NHANES III.

		HR (95% CI)	
	N cancer death/*N* total	Model 1[1]	Model 2[2]
Men			
Leptin (μg/L)			
Low	49/909	1.0 (Reference)	1.0 (Reference)
Moderate	71/962	0.87 (0.51–1.49)	0.75 (0.43–1.35)
High	79/964	0.86 (0.50–1.47)	0.60 (0.26–1.33)
P_{trend}		0.60	0.22
CRP (mg/L)			
Low	195/2047	1.0 (Reference)	1.0 (Reference)
Moderate	56/596	1.33 (0.82–2.18)	1.31 (0.85–2.04)
High	34/192	2.98 (1.57–5.64)	2.90 (1.52–5.53)
P_{trend}		0.003	0.002
$P_{interaction}$ leptin and CRP		0.13	0.13
Women			
Leptin (μg/L)			
Low	54/1081	1.0 (Reference)	1.0 (Reference)
Moderate	71/1096	1.19 (0.74–1.91)	0.81 (0.51–1.30)
High	61/1128	0.93 (0.57–1.51)	0.40 (0.24–0.68)
P_{trend}		0.80	0.0007
CRP (mg/L)			
Low	103/2027	1.0 (Reference)	1.0 (Reference)
Moderate	58/914	1.41 (0.84–2.38)	1.19 (0.67–2.11)
High	25/364	1.10 (0.61–1.99)	0.86 (0.45–1.63)
P_{trend}		0.28	0.99
$P_{interaction}$ leptin and CRP		0.72	0.81

[1]Adjusted for age (continuous) and waist circumference, race/ethnicity, poverty-to-income ratio (PIR), tobacco smoking, alcohol consumption, and vigorous physical activity.

[2]Adjusted for age (continuous) and waist circumference, race/ethnicity, PIR, tobacco smoking, alcohol consumption, vigorous physical activity, body mass index (continuous) and waist circumference (continuous), and waist circumference.

Finally, to account for competing risks, we ran Fine and Gray regression to estimate cumulative mortality of cancer over time with levels of leptin or CRP as the predictor variable and deaths from major cardiovascular diseases and other causes as competing outcomes. The analysis was adjusted for all potential confounders including BMI and waist circumference. Men with higher CRP were shown to have higher cumulative mortality from cancer over time ($P = 0.009$), whereas no association for serum leptin was found ($P_{trend} = 0.17$). In women, serum CRP was not suggested to correlate with cancer mortality ($P = 0.59$). On the other hand, higher cumulative mortality from cancer was noted in women with higher serum leptin ($P = 0.006$). Therefore, our results from the competing risk analysis corroborated our findings from Cox regression models.

Discussion

This study was based on a nationally representative sample of the U.S. population. We observed a protective effect of leptin against cancer death in women and higher cancer death with increased CRP in men. No marked interaction between leptin and CRP was found. Similar associations were observed when competing risk analyses with deaths from major cardiovascular diseases and other causes as competing outcomes were employed.

Proposed mechanisms linking leptin and carcinogenesis mostly suggest that higher leptin exposure increases predisposition to the disease [3]. The long isoform of leptin receptor (Ob-R) is similar to a type I cytokine receptor, with an ability to activate downstream JAK/STAT signaling pathway, a known transcription activator for genes involved in cell proliferation, survival, angiogenesis, and metastasis [27]. Furthermore, the activation of ObR may lead to phosphorylation of insulin receptor substrate (IRS-1), initiating activation of PI3K/Akt pathway, which is also important in carcinogenesis [28]. Besides directly eliciting cancer-related signaling, leptin also displays proinflammatory properties [29]. Inflammation may also promote cancer by activation of signaling molecules including STAT3 and NF-κB [30]. Despite suggestive experimental findings, there is limited observational evidence documenting the importance of leptin-inflammation interplay in cancer incidence or mortality.

Table 3. Sex-specific associations of serum leptin and C-reactive protein (CRP) with cancer death in the NHANES III, stratified by obesity status. All models were adjusted for age, race/ethnicity, poverty-to-income ratio (PIR), tobacco smoking, alcohol consumption, and vigorous physical activity.

	General obesity[1]		Abdominal obesity[2]	
	Not obese	Obese	Not obese	Obese
Men				
N cancer death/*N* total	145/2177	43/582	114/1965	74/794
Leptin (μg/L)				
Low	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Moderate	0.85 (0.50–1.44)	1.18 (0.22–6.40)	0.91 (0.47–1.76)	4.09 (0.88–19.10)
High	0.61 (0.24–1.53)	1.56 (0.35–6.94)	0.64 (0.20–1.99)	2.71 (0.64–11.45)
P_{trend}	0.28	0.56	0.49	0.59
$P_{interaction}$ leptin and obesity		0.52		0.43
CRP (mg/L)				
Low	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Moderate	0.99 (0.52–1.89)	1.48 (0.41–5.40)	1.06 (0.57–1.97)	1.33 (0.63–2.79)
High	3.62 (1.86–7.04)	0.59 (0.13–2.72)	2.22 (0.97–5.05)	3.43 (1.03–11.48)
P_{trend}	0.004	0.89	0.17	0.07
$P_{interaction}$ CRP and obesity		0.29		0.64
$P_{interaction}$ leptin and CRP	0.39	0.09	0.11	0.08
Women				
N cancer death/*N* total	112/2235	67/963	52/1438	127/1760
Leptin (μg/L)				
Low	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Moderate	0.76 (0.44–1.32)	1.77 (0.20–16.16)	1.06 (0.49–2.30)	1.24 (0.64–2.41)
High	0.30 (0.14–0.65)	0.65 (0.08–5.08)	N/A	1.07 (0.55–2.07)
P_{trend}	0.003	0.01	0.15	0.09
$P_{interaction}$ leptin and obesity		0.63		0.41
CRP (mg/L)				
Low	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Moderate	1.32 (0.66–2.63)	0.89 (0.33–2.36)	1.00 (0.47–2.11)	1.29 (0.70–2.36)
High	0.65 (0.25–1.68)	0.92 (0.43–1.94)	N/A	1.14 (0.62–2.07)
P_{trend}	0.78	0.89	0.40	0.63
$P_{interaction}$ CRP and obesity		0.83		0.35
$P_{interaction}$ leptin and CRP	0.77	0.37	0.92	0.46

[1]Adjusted for waist circumference (continuous).
[2]Adjusted for body mass index (continuous).

Findings from population-based studies for the link between leptin and cancer are scarce. Some evidence suggests a positive association between prediagnostic serum leptin and risk of cancer for colorectal [31], breast [8], prostate [32], and endometrial cancer [33], as well as renal cell carcinoma [4], but results are contradictory [34, 35]. In a large nested case–control study based on the European Prospective Investigation into Cancer and Nutrition (EPIC), no association was reported between serum leptin and risk of colorectal cancer regardless further adjustment for BMI (RR: 0.85 (95% CI: 0.56–1.29) for the highest quintile compared to the lowest; $P_{trend} = 0.76$) [10]. We found a lack of association between leptin and cancer death, whereas CRP was positively associated to cancer death in men. The inverse association for leptin, which was not observed in previous studies such as the EPIC Study focusing on colorectal cancer [10] might be attributed to the use of cancer mortality as an outcome instead of cancer incidence. Therefore, it is possible that leptin, despite being weakly associated to cancer incidence, may reflect susceptibility for fatal malignancies.

With respect to obesity, leptin and inflammation have gained increasing interest with regards to their potential implications in cancer development [12]. Leptin resistance may occur in obesity [36], where higher levels of leptin follow. Interestingly, CRP has been identified as one of the major serum leptin-interacting proteins (SLIPs) which may worsen leptin resistance [37] and support a biological interaction between leptin and CRP in the context of diseases. In this study, a nearly statistically significant interaction between leptin and CRP was observed in men with abdominal obesity. Romero-Corral and colleagues [21] stated that such interaction occurs when assessing cardiovascular disease, resulting in a weaker association between CRP and cardiovascular disease after adjustment for leptin. Nevertheless, we did not observe any alteration

in our findings after leptin and CRP were both included in the same analysis, thus suggesting minimal interaction between serum leptin and CRP with respect to cancer mortality as an outcome.

In many studies including ours, serum concentrations of leptin are positively correlated with that of CRP regardless of obesity [38, 39]. However, we observed different effects between leptin and CRP levels on cancer mortality. This may signify differential roles between leptin and CRP in the scope of cancer, which require further biological investigations. From the competing risk analysis where we took into account competing outcomes, it was further suggested the inverse association between serum leptin and cumulative mortality from cancer in women, and a positive association for CRP in men. Our findings therefore indicate that the associations between leptin or CRP and cancer may be unique and do not resemble the additive effects observed with cardiovascular disease [21]. This interesting observation may suggest different biological pathways linking leptin and inflammation with cancer, which may involve sex-specific biological and environmental factors. Such observations thus call for further investigations to assess specific cancers and relevant mechanistic approaches.

This strength of this study is its generalizability following the use of nationally representative data of the U.S. population. We were able to adjust for potential confounders and stratify by overweight status. To our knowledge, this is the first study investigating the interaction between leptin and CRP in relation to cancer in the population. A limitation of this study is that there was no information on cancer incidence, so that we were only able to assess these markers in relation to cancer mortality. In the NHANES, information on causes of death was collected by means of probabilistic matching [19]. Although we only selected those considered to have eligible mortality status, potential misclassification may have occurred. Low number of cases also hampered our stratification analyses, and therefore future studies with sufficient number of cases are necessary to further investigate this topic. Additionally, our analyses relied on a single measurement so that it may be prone to measurement error and within-person variation. The laboratory methods used for CRP measurement at the time the NHANES III was conducted were unable to perform a high sensitivity assay of this marker. Nevertheless, serum CRP in the NHANES III population was reported to be associated to CRP-related genetic variation [40], justifying the usefulness of this marker despite its limitation in quantitatively measuring low levels of CRP. Finally, abnormal levels of leptin and CRP may occur secondary to cancer which may result in reverse causation. We have excluded participants with cancer at baseline, however, residual confounding may have occurred.

Conclusion

Our study showed that leptin may be inversely associated with cancer mortality in women, and CRP corresponded with higher risk for cancer death in men. Interaction between CRP and leptin is likely to be minimal in the study on cancer mortality, unlike previous evidence suggested in cardiovascular disease. It is imperial for further studies to address the discrepancies in effects on cancer between adipokines and inflammatory markers in order to fully comprehend the mechanism linking obesity-related features and carcinogenesis. Furthermore, the differential associations with cancer death between men and women may point toward their potential use in future risk modification strategies targeting mortality from cancer.

Conflict of Interest

No conflict of interest declared.

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Appendix B2. Associations of C-reactive protein, granulocytes and granulocyte-to-lymphocyte ratio with mortality from breast cancer in non-institutionalized American women. Poster presented at the 2014 ESMO IMPAKT Breast Cancer Conference. Awarded with an ESMO Travel Grant.

CRP and granulocyte-to-lymphocyte ratio as predictors of breast cancer death in non-institutionalized American women

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Background

- Inflammation may promote the development and progression of cancer, but its role in breast cancer (BCa) is unclear
- Many studies used serum C-reactive protein (CRP) as a marker of inflammation; however, it is affected by metabolic factors such as obesity
- Absolute granulocyte count (AGC) and its relative ratio to lymphocyte (G/L ratio) have been linked to cancer prognosis but so far they have only been studied in clinical settings
- Assessing these markers in relation to BCa death in a population based-study may enable a better insight into how inflammation is linked to BCa

Aim

To assess the association between inflammation and BCa death using serum inflammatory markers: CRP, AGC and G/L ratio, while taking into account obesity and BCa risk factors

Data

- The National Health and Nutrition Examination Survey III (NHANES III; Figure 1) linked with mortality follow-up through 31 December 2006
- A cohort of 8,184 women aged 20+ years with baseline measurements of serum CRP, AGC and G/L ratio

Methods

- Cox proportional hazards regression to assess CRP, AGC and G/L ratio in relation to risk of BCa death and death from all cancer, cardiovascular disease and all causes
- Multivariable models were adjusted for age, race/ethnicity, smoking, alcohol consumption, vigorous physical activity, aspirin use, history of diabetes and cardiovascular disease. Models were also adjusted for menopausal status, age at menarche, use of contraceptive pills and history of cancer when analysing risk of BCa and all cancer death. Furthermore, an additional adjustment for body mass index (BMI) were performed for all models to account for obesity.
- Stratified analyses based on overweight and menopausal status

Results

	Weighted mean/proportion				
	Breast cancer death (n = 59)	All cancer death (n = 366)	Cardiovascular death (n = 868)	All death (n = 1877)	Alive (n =6307)
Age (years)					
Mean (SD)	52.68 (3.43)	61.10 (1.31)	71.88 (0.74)	67.90 (0.71)	41.06 (0.40)
Mean follow-up (months)					
Mean (SD)	102.54 (9.27)	99.09 (4.42)	99.05 (2.59)	101.83 (1.94)	179.66 (2.86)
Race = Ethnicity					
Non-Hispanic white	74.66	82.22	84.95	83.23	75.03
Non-Hispanic black	13.81	11.20	9.89	10.59	11.16
Mexican American	3.42	2.75	1.90	2.25	5.17
Other	8.12	3.83	3.26	3.93	8.63
Alcohol consumption					
Never	61.84	65.08	75.99	72.21	49.83
Up to once a week	12.55	18.11	12.34	13.72	22.20
2-3 times a week	10.14	5.73	2.43	4.09	13.31
4-6 times a week	5.99	3.84	2.86	3.17	9.52
Daily or more	9.48	7.24	6.38	6.81	5.14
Cigarette smoking					
Never	58.38	37.98	58.71	50.21	55.66
Former	8.69	28.68	24.94	26.15	19.19
Current	32.93	33.35	16.35	23.64	25.15
Vigorous Physical activity	11.25	18.18	27.73	23.91	6.28
BMI (kg/m²)					
< 18.50	6.53	4.07	3.76	4.96	3.16
18.50-25	12.66	31.13	35.69	34.27	47.43
25.00-30	37.59	33.82	32.00	32.36	25.18
≥ 30.00	43.23	30.98	28.55	28.42	24.24
Menopausal status					
Premenopause	36.24	14.73	5.09	9.28	62.55
Perimenopause	7.14	3.84	0.99	1.58	5.85
Postmenopause	56.62	81.43	93.92	89.14	31.61
Age at menarche (years)					
Mean (SD)	13.07 (0.45)	12.71 (0.11)	13.12 (0.07)	13.00 (0.06)	12.74 (0.03)
Age at first childbirth (years)					
Mean (SD)	22.89 (0.64)	22.53 (0.34)	22.82 (0.21)	22.67 (0.17)	22.26 (0.12)
History of breastfeeding	41.46	56.86	66.26	61.83	53.52
Parity					
0	9.48	6.60	4.71	4.06	8.27
1	19.82	15.41	17.65	16.50	20.59
2+	70.70	77.99	77.64	79.44	71.14
History of oral contraception	66.69	37.70	14.17	21.31	69.63
History of cancer	21.77	15.26	12.79	12.03	3.66
History of diabetes	5.07	11.42	17.88	16.32	3.97
History of cardiovascular disease	1.25	3.04	2.01	2.14	0.37
Aspirin use	43.94	37.04	44.88	39.97	34.36
CRP (mg/L)					
Mean (SE)*	11.23 (1.64)	10.94 (1.01)	12.59 (0.88)	12.21 (0.66)	10.86 (0.03)
Undetectable (< 2.2)	55.66	57.67	55.82	55.77	67.60
Intermediate (2.2-10)	22.88	23.97	26.32	26.28	23.77
Clinically raised (≥ 10)	21.46	18.36	17.86	17.95	8.63
AGC (10^3 cells/L)					
Mean (SE)	5.14 (0.46)	4.78 (0.16)	4.80 (0.08)	4.82 (0.06)	4.55 (0.04)
G/L ratio					
Mean (SE)	2.50 (0.23)	2.37 (0.11)	2.43 (0.05)	2.39 (0.04)	2.11 (0.02)

*Clinically detectable CRP (≥ 2.2 mg/L)

Table 1. Weighted baseline characteristics of study participants by cause of death

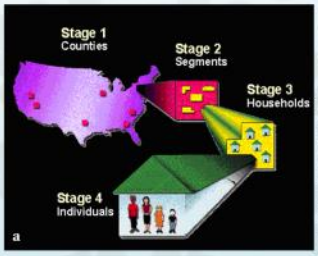

Figure 1. NHANES III sampling. The NHANES III used a multi-cluster, stratified sampling design, and a sample weight was assigned to each sample person (a). All persons participated in an interview conducted at home and an extensive physical examination, which included a blood sample taken in a mobile examination centre (b). (*www. CDC.gov*)

- During a mean follow-up time of 167 months, a total of 1,877 women died: 366 of cancer, including 59 who died of BCa, and 868 of cardiovascular disease (Table 1)
- G/L ratio was associated with risk of BCa death despite further adjustment for BMI (HR: 3.21 (95% CI: 1.44-7.18) for every log increase in G/L ratio)
- No association of CRP and AGC with risk of BCa death was observed (Figure 2)
- Sensitivity analyses excluding those with self-reported history of cancer at baseline and follow up < 3 years yielded similar results

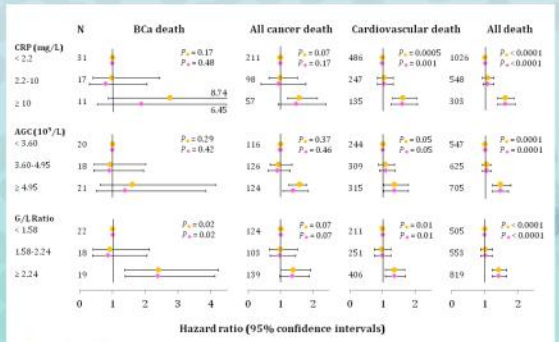

Figure 2. Hazard ratios for death from BCa, overall cancer, cardiovascular disease and all causes by clinical cut offs of CRP and tertiles of AGC and G/L ratio

- Effect modification by overweight status was observed for CRP and G/L ratio in relation to BCa death (Figure 3)
- Stratified analysis by menopausal status showed that only clinically detectable CRP was positively associated with BCa death (HR: 2.25 (95% CI: 1.43-3.55) for every log increase in CRP) in postmenopausal women

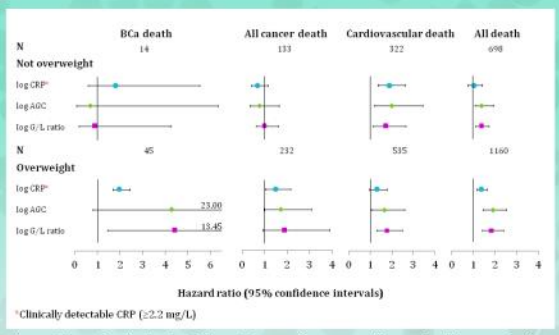

Figure 3. Hazard ratios for death from BCa, overall cancer, cardiovascular disease and all causes for log-transformed values of CRP, AGC and G/L ratio, stratified by overweight status (BMI </≥ 25 kg/m²)

Conclusion

- Inflammation is associated with an increased risk of death from BCa
- G/L ratio was strongly associated with risk of BCa death, and its use in addition to CRP may be important in assessing fatal BCa risk
- Lack of association between CRP and BCa in previous studies may be accounted for by potential effect modifiers such as obesity and menopausal status
- All three markers were associated with risk of death from cardiovascular disease and all causes, indicating the importance of addressing other causes of death when looking into the association between inflammatory markers and BCa mortality

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